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ABSTRACT

MODERN VIEWS ON DENTIN STRUCTURE AND ITS MORPHO-FUNCTIONALITY AS LIVING MATTER

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Background. During significant mechanical loading on teeth, dentin plays a crucial role in preserving and maintaining their function due to its unique architectural features.

The objective of the research was to study the state of the structural-functional pattern of dentin (dentinal tubules, odontoblast processes, and fibrillar apparatus) in human teeth at different distances from the pulp chamber.

Methods. In patients aged 18-20 years, 30 intact teeth extracted for orthodontic indications were studied. Immediately after extraction, the crowns were separated from the roots and divided along the tooth axis and in the mesiodistal direction, followed by immersion in Karnovsky's fixative solution. The samples were washed in cacodylate buffer, demineralized, dried, impregnated with epoxy resin in a VUP-5M vacuum apparatus (VO "SELMI", Sumy, Ukraine), polished and examined under a MICROmed Evolution ES-4140 light optical microscope with a 5 MP digital camera ("Mikromed", Ukraine). Some samples, after freeze-drying by avoiding the critical point transition method, were glued with electrically conductive adhesive onto copper tables and covered with a 20 nm layer of chemically pure aluminum [999 grade]. Sections were prepared using standard methods for scanning electron microscopy and examined in a scanning electron microscope ("JEOL-25M-T220A" (Tokyo, Japan)).

Results. The results of light optical examination indicate the existence of a structural-functional pattern of dentin characteristic of all human teeth. This pattern includes 3 elements: dentinal canaliculus, dentinal tubule, and fibrillar apparatus. Together they define four bands of dentin: black, dark, gray, and light. In turn, their intensity and width depend on the presence or absence of the other two constituent elements of the structural-functional pattern of dentin in the dentinal canaliculus. The boundary between each band also depends on whether there is a

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dentinal tubule with a complete fibrillar system in the dentinal canaliculus. We have shown that numerous microfibers of dentinal canaliculi form a dense fibrillar network - a source of hydrophilic proteins, which is the basis for creating parietal gaps that determine the capillary tension forces necessary to maintain centrifugal transport of pulpal lymph. The complex of constituent elements of the structural-functional pattern of dentin determines the degree of mineralization of teeth and, accordingly, the effectiveness of treatment of carious lesions and remineralization therapy measures, which affects the restoration of the structure of its individual elements.

Conclusion. The presented material deepens knowledge about the structure of dentin, demonstrates the composition of its structural-functional pattern capable of creating capillary tension forces to support centrifugal transport of pulpal lymph. Constant circulation of pulpal lymph is the basis of living matter, which not only does not contradict the classical model of structural-functional relationships but is also organically substantiated by morphological research methods.

Keywords: human tooth, dentin, structural-functional pattern, dentinal bands, fibrillar apparatus, dentinal canaliculus, dentinal tubule, morphological studies, SEM studies.

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СУЧАСНІ ПОГЛЯДИ НА СТРУКТУРУ ДЕНТИНУ ТА ЙОГО МОРФО-ФУНКЦІОНАЛЬНІСТЬ ЯК ЖИВОЇ МАТЕРІЇ

Вступ. При значному механічному навантаженні на зуби в збереженні та підтримці їх функції вирішальну роль відіграє дентин завдяки його унікальним архітектурним особливостям.

Метою дослідження було вивчення стану структурно-функціонального патерну дентину (дентинних канальців, відростків одонтобластів і фібрилярного апарату) в зубах людини на різній відстані від пульпової камери.

Матеріали та методи. У пацієнтів віком 18-20 років вивчали 30 інтактних зубів, видалених за ортодонтичними показаннями. Відразу після екстракції зуба відділяли від коренів коронки та розділяли їх вздовж осі зуба та в мезіодистальному напрямку з подальшим зануренням у розчин фіксатора за Карновським. Взірці промивали в какодилатному буфері, демінералізували, висушували, просочували епоксидною смолою у вакуумному приладі ВУП-5М (ВО «SELMI», Суми, Україна), шліфували та досліджували на світлооптичному мікроскопі MICROmed Evolution ES-4140 з цифровою камерою 5 МП («Мікромед», Україна). Частину взірців після ліофілізації заморожуванням методом уникнення переходу критичної точки наклеювали електропровідним клеєм на мідні столики і покривали шаром хімічно чистого алюмінію [проба ^{999}] товщиною 20 нм. Шліфи готували загальноприйнятим для скануючої електронної мікроскопії методом і досліджували в сканувальному електронному мікроскопі (“JEOL-25M-T220A” (Токіо, Японія)).

Результати. Результати світлооптичного дослідження свідчать про існування структурно-функціонального патерну дентину, характерного для всіх зубів людини. До складу цього патерну

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входять 3 елементи: дентинний каналець, дентинна трубочка та фібрилярний апарат. В сукупності вони визначають чотири смужки дентину: чорну, темну, сіру і світлу. В свою чергу їх інтенсивність і ширина залежать від наявності чи відсутності в дентинному канальці двох інших складових елементів структурно-функціонального патерну дентину. Межа між кожною смужкою залежить також від того, чи є в дентинному канальці дентинна трубочка з повноцінною фібрилярною системою. Нами показано, що численні мікроволокна дентинних канальців утворюють густу фібрилярну сітку – джерело гідрофільних білків, яка є основою для створення пристінкових щілин, що визначають сили капілярного натягу, необхідні для підтримки відцентрового транспорту пульпової лімфи. Комплекс складових елементів структурно-функціонального патерну дентину визначає ступінь мінералізації зубів і, відповідно, ефективність лікування каріозних уражень зубів і проведених заходів ремінералізуючої терапії, що впливає на відновлення структури його окремих елементів.

Висновки. Представлений матеріал поглиблює знання про структуру дентину, демонструє склад його структурно-функціонального патерну, здатного створювати сили капілярного натягу для підтримки відцентрового транспорту пульпової лімфи. Постійна циркуляція пульпової лімфи є основою живої матерії, що не тільки не суперечить класичній моделі структурно-функціональних взаємовідносин, але й органічно обґрунтовується морфологічними методами дослідження.

Ключові слова: зуб людини, дентин, структурно-функціональний патерн, дентинові смужки, фібрилярний апарат, дентинний каналець, дентинна трубочка, морфологічні дослідження, СЕМ-дослідження.

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INTRODUCTION

The pandemic caused by the SARS-CoV-2 coronavirus has had a significant impact on dental morbidity, resulting in a substantial decline in the oral health index. According to WHO experts [1], approximately 50% of the world's population suffers from oral diseases, with untreated dental caries being one of the five most common non-communicable diseases. Therefore, studies aimed at studying the stability indicators of hard dental tissues may be valuable in understanding the relationship between dental structure and dental functions. A detailed study of the microstructure and permeability of hard dental tissues is of great importance for the modern development of effective infiltration and adhesive materials for the treatment of hard dental tissues [2, 3].

It is known that teeth are exposed to intense mechanical and chemical influences, as well as to strong microbial colonization. At high levels of mechanical stress, teeth can remain healthy, which indicates the presence of mechanical adaptation mechanisms in their structure to counteract constant stress [4]. Dentin plays a crucial role in the mechanical support of tooth function, particularly due to its flexibility, strength, and rigidity, which allow teeth to withstand mechanical loads without being destroyed. A number of studies focus specifically on dentin's mechanical properties. Thus, the publication by Kinney JH. et al. [5] presents a review of studies performed over 50 years. A critical assessment of these studies showed that the elastic properties of dentin are anisotropic, and large coefficients of variation of strength indicate a possible distribution of defects in dentin samples. In an experimental study, Soukup JW. et al. [6] demonstrated significant anisotropy and spatial variation in the modulus of elasticity and hardness of canine dentin, which is due to the fraction of its area from the superficial to the deep dentin layers.

The study of dentin microstructure features showed that the mechanical behavior of dentin can be

attributable to the content of minerals and organic components [6]. It is known that dentin, a viable mineralized tissue containing collagen, constitutes the bulk of the tooth and has a chemical composition similar to bone [7, 8]. According to physiological and anatomical studies, dentin is a complex structure composed of collagen fibers, nanocrystalline hydroxyapatite, and numerous tubular networks [9]. The main mass of the tooth is circumpulpal dentin, which includes intertubular and peritubular dentin. Goldberg M. et al. [10] considered the features of different types of dentin in mammalian teeth. They found that the outer layers included mantle dentin, Tomes' granular layer, and Hopewell-Smith's hyaline layer. Deang JF. et al. [11] studied the chemical composition of enamel and dentin of the teeth of the sheepshead *Archosargus probatocephalus* and the microstructural characteristics of the density and area of dentinal tubules. The results of the authors' study using energy-dispersive X-ray spectroscopy showed that the dentin of sheepshead teeth had a significant microhardness that increases closer to the tooth surface, which could be a result of the significant fluoride content in the dentin. Furthermore, odontoblasts are known to synthesize and secrete a unique set of non-collagenous proteins – the collagen matrix; in particular, three specific dentin proteins have been recognized as produced by odontoblasts and potentially initiating the formation and growth of apatite crystals and mineralization [12, 13]. The study identified three stages of dentinogenesis [10]. Thus, matrix vesicles participate in the early formation of dentin; collagen and some proteoglycans participate in the formation of predentin, which is later transformed into intertubular dentin; and the formation of peritubular dentin is carried out due to the secretion of non-collagen molecules of the dentin extracellular matrix. Dentin sialophosphoprotein, the most abundant non-collagenous dentin protein, is crucial for proper mineralization of tooth dentin [14].

It is known that topographically, the peripheral part of the tooth pulp contains odontoblasts – postmitotic cells responsible for the formation and maintenance of dentin; the odontoblastic processes, dentin fibers (DFs), are located in the dentinal tubules (DTs) [15, 16]. Odontoblast processes were first described by Tomes J. [17], who considered them as a cytoplasmic branch of the dental pulp odontoblast located inside the DTs. According to Tomes, odontoblastic processes secrete all protein and non-protein components of dentin that participate in its primary and secondary mineralization. After Tomes first described odontoblastic processes, they were found to be a highly complex biological structure located within the dentinal tubule. Peritubular dentin surrounds this structure, and intertubular dentin encompasses both dentinal tubules and dentin fibers [18]. The idea of the dentin structure has changed over the years. Thus, in 1962, Johansen E. and Parks HF. [19] identified the role of thin lamellar sheet-like membranes as peculiar stabilizers of the central position of the DFs in the dentinal tubule throughout the entire thickness of the dentin. Odontoblastic processes, Tomes fibers, are directed towards the dentin-enamel junction of the anatomical crown of the tooth and are a cellular component of the dentin structure [20]. Williams C. and Wu Y. [21] used light microscopy to compare the density of dentinal tubules and the distances between them in different layers of dentin. They mapped the distribution of dentinal tubules in the crowns of adult incisors and molars to establish the structural and functional features of dentin.

The unique architectural features of dentin, which has a peculiar micromorphology and the ability to regulate mechanosensory functions through mechanosensitive ion channels, contribute to the sensory perception of external stimuli and act as a protective barrier in the dentin-pulp complex [4]. Saberi E. et al. [22] conducted an experimental study of morphometric parameters of dental pulp in a sheep model after mechanical pulp exposure and restoration with zinc oxide-eugenol. They demonstrated changes in some morphometric parameters of dental pulp in response to mechanical exposure and restoration. A comparative assessment of the density of dentinal tubules and the percentage of dentin area occupied by them in the vault and the floor of the pulp chamber was carried out by Kontakiotis EG. et al. [23] on intact third molars of the lower jaw of a human subject. The results showed that the vault of the pulp chamber had a higher density of dentinal tubules and a larger percentage of dentin area occupied by the openings of the tubules compared to the floor of the pulp chamber. The results of other studies indicate the presence of a complex microfibrillar network that, within the DTs, connects to the DF membrane and, apparently, serves as a support system for the dentinal tubule to maintain and support the odontoblastic process [24]. According to a number of studies, DFs are located only in the predentin zone [12], or only in the inner third of the entire length of the DTs [24, 25], or in the outer third of its length, reaching the dentin-enamel junction in the coronal and root parts of the tooth [26].

As shown in the data we have provided, the interpretation of the structure of dentinal tubules and fibers at different distances from the pulp chamber remains ambiguous. The obtained data emphasize the need for detailed characterization of dentin substrates, which served as the basis for conducting our own study.

The study aims to assess the structural and functional patterns of dentin (dentinal tubules, odontoblastic processes, and fibrillar apparatus) in human teeth at varying distances from the pulp chamber.

MATERIALS AND METHODS

The studies were approved by the Bioethics Commission of the I. Ya. Horbachevsky Ternopil National Medical University (protocol No. 75 dated 01.11.2023) and were conducted in accordance with the bioethical principles set out in the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki of the World Health Association on Ethical Principles of Medical Research Involving Human Subjects (1964–2000), and the Universal Declaration on Bioethics and Human Rights (UNESCO). Written informed consent was obtained from all the participants.

In patients aged 18–20 years, 30 intact teeth removed for orthodontic indications were examined. Immediately after tooth extraction, the crowns were separated from the roots by making a groove along the cementoenamel junction with a tungsten carbide bur and a high-speed water-cooled tip. The splitting was done with a dental spatula, which served as a kind of chisel, and a dental hammer. The crowns were divided into two halves in the mesiodistal direction, avoiding their lateral displacement (Fig. 1a). To make a transverse cut, the samples were prepared by the rupture method in a special device, for which a force was applied to the tooth along its axis (Fig. 1b). Since the walls of the DFs after lyophilization are very fragile, the samples were immediately immersed in Karnowski's fixative solution at 4°C for 24 h. The samples were then washed in cacodylate buffer (pH = 7.4) and demineralized in 5% aqueous nitric acid solution. After drying, the samples were impregnated with epoxy resin in the VUP-5M vacuum device (OJSC “SELMI”, Sumy, Ukraine) and polished with paper discs with diamond paste (diameter of grinding abrasive = 20 μm). The slices were mounted on glass slides, impregnated with a 20% silver nitrate solution according to the generally accepted method [27], and examined with a MICROmed Evolution ES-4140 light-optical microscope with a 5 MP digital camera (Micromed, Ukraine). Part of the samples was subjected to freeze-drying by the method of avoiding the critical point transition, then they were glued with electrically conductive glue onto copper tables, covered with a 20 nm layer of chemically pure aluminum [999 grade], and viewed in JEOL-25M-T220A scanning electron microscope (Tokyo, Japan).

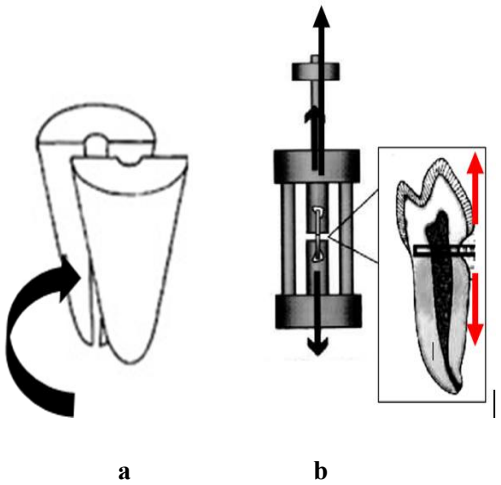
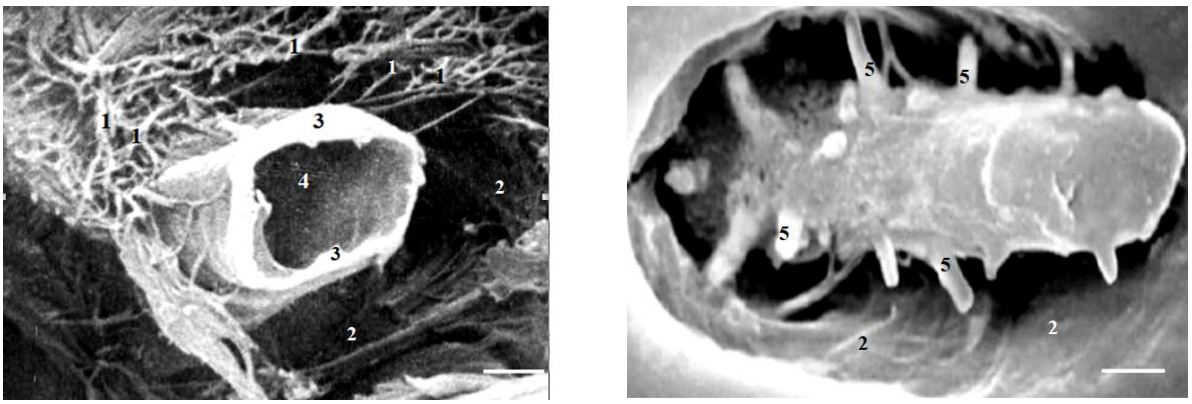


Figure 1 – Schematic representation of the preparation of a longitudinal cut (a) and a transverse rupture (b) of a tooth

RESULTS AND DISCUSSION

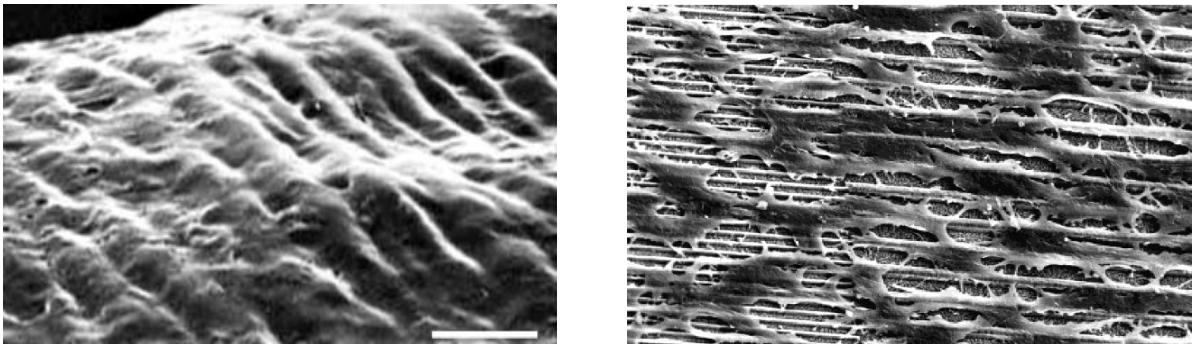
The structural features of parapulpal dentin did not differ from those previously reported by us [25]. In the inner unmineralized dentin, predentin, each DT contained only one DF, connected to the walls of the dentinal tubule by numerous thin microfibrillar structures that form a dense network (Fig. 2a). In some specimens, the central lumen of the dentinal fiber was obturated, but well-preserved anchoring fibrils were visible on its walls (Fig. 2b). In the inner third of dentin, one end of the microfibril was always fixed to the DF wall, and the other end was in contact with the DT wall. The spaces between these microfibers were only 0.3-0.5 microns, and the microfibers themselves were so numerous that they occupied most of the DTs, forming a dense network. Such a fibrillar network is the basis for creating microscopic wall gaps that determine capillary tension forces. According to the data of Okushko V. R. [28], this force is a necessary condition for maintaining the centrifugal transport of pulp lymph, due to which a pressure gradient is maintained in the hydrodynamic system of hard tissues and favorable conditions are formed for the operation of the “dental pump” – the hydraulic system of the tooth, which is necessary for the nutrition, maintenance and restoration of the properties of tooth hard tissues.

In other areas of dentin, microfibrils merge, become wider, and form broad flattened structures (biofilms), and often appear as a homogeneous (Fig. 3a) or fragmented film material (Fig. 3b). SEM study illustrates the general appearance of a sheet-like continuous biofilm covering the dentinal tubules together with dentinal fibers on the dentinal surface. Fragmentary biofilm material is also visualized between adjacent dentinal tubules and dentinal fibers.



a b

Figure 2 – General view of the structures of internal unmineralized dentin: a – framework structure of the fibrillar network (1); walls of the dentinal tubule (2); central position of the dentinal fiber (3); wide cavity in the DF (4); anchor fibrils (5); b – preserved anchor fibrils (5) in the central lumen of the DF. Method: SEM, sublimation by the critical point transition method. Accelerating voltage 15 kV; original magnification: a × 1750; b × 1500; scale bar = 20 μm



a b

Figure 3 – General view of a sheet-like continuous biofilm (a) and fragmented biofilm material (b). Method: SEM. Accelerating voltage 10 kV; original magnification: a × 3500; b × 2000; scale bar = 30 μm

In the inner third of dentin, the DFs were located in the center of the DTs and were always in close contact with the microfibrillar network. Most often, these microfibrils were directed from the surface of the DFs to the wall of the DTs at an acute angle; in the absence of DFs, these microfibrils were attached to both opposite inner surfaces of the DTs (Fig. 4). This state corresponds to a general biological law: in the presence of a functional structure, a structural-functional pattern is formed, and in the absence of at least one element of such a pattern, the need for a supporting apparatus disappears, and such a structure can manifest itself only in the form of individual rudiments. SEM study demonstrates the general appearance of an incomplete structural-functional pattern of dentin, in which, in the absence of a dentinal fiber, collagen microfibrils are fixed to the opposite walls of the dentinal tubule. Other dentinal tubules are free of the fibrillar component and do not contain dentinal fibers.

The results of our studies on the presence of the own membrane in DFs, represented by a limiting plate, *the lamina limitans* (Fig. 5), and is traced along the predentin-dentin and enamel-dentin junctions, are consistent with the data of other authors [29, 30]. The

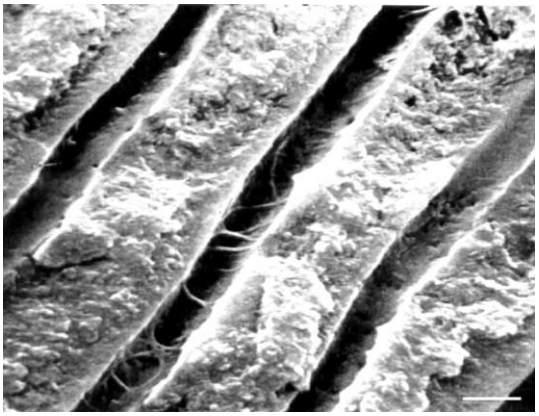


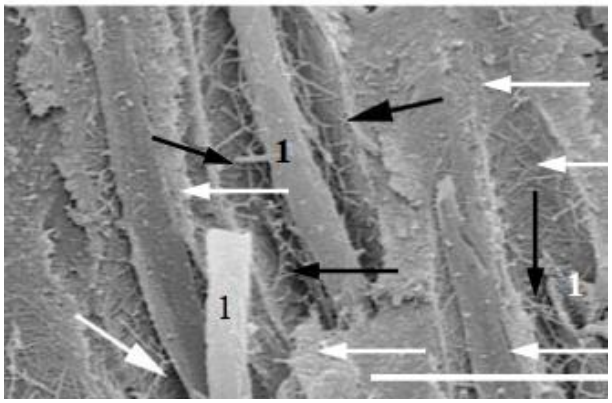
Figure 4 – General view of the incomplete structural and functional pattern of dentin. Method: SEM, sublimation by the critical point transition method. Accelerating voltage 15 kV; original magnification × 1750; scale bar = 20 μm

SEM illustration presents a general view of the terminal sections of the dentinal fibers, their fibrillar apparatus, and the biofilm around them in the form of *the lamina limitans*, which are often connected to adjacent dentinal tubules. These structures are seen along the entire length of the DTs and are found even in the area of the enamel-dentine junction (Fig. 5b). Individual dentinal tubules contain only remnants of dentinal fibers, and the preserved dentinal fibers have terminal thickenings that contact the basal layer of enamel. The *lamina limitans* is clearly visualized and connects to a similar structure of the adjacent dentinal fiber. In this case, the anchor fibers start from the *lamina limitans* and penetrate into the lateral tubules, never contacting neighboring DTs (Fig. 5c).

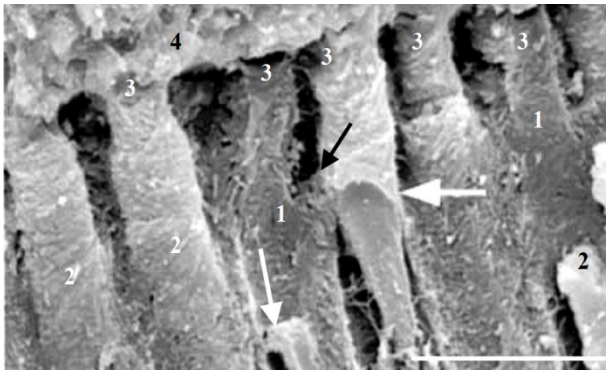
We did not observe any boundaries between fibrillar or sheet-like structures and peritubular dentin. The microfibril base is attached to the DT wall and forms a continuous network structure of the dentin and DF surface. In different areas of dentin, the length, quantity, and diameter of microfibrillar material vary widely. The greatest amount of this material is in the first ¾ of the DT length, which determines the dark dentin stripe on longitudinal slides of teeth impregnated with a silver nitrate solution (Fig. 6).

In a previously published article [25], we reported that microfibrillar and sheet structures were very rare, especially on DFs located in the outer third of dentin, which determines the presence of a gray dentin stripe on longitudinal slides of teeth impregnated with silver salts. We established that when impregnated with a silver nitrate solution, dentin had several separate zones or stripes, which are naturally found in the dentin of any tooth. Each of these stripes has its own characteristics, due to the nature of the structural and functional pattern of dentin, which are visualized by the method of impregnation with silver salts at the light-optical level. Thus, in the zone of dense secondary dentin around the DFs, a dense network of collagen fibers is observed, which consists of a number of proteins that absorb silver ions well; therefore, on the micrographs, this zone has a rich dark colour. In addition, these proteins have pronounced hydrophilic properties contributing to the creation of high osmotic pressure, which, through a system of mesh microspaces, generates a suction force of capillary tension for the mineral-rich intercellular fluid – pulp lymph [28]. This intercellular fluid is transported in a centrifugal direction – from the pulp chamber to the periphery, to the enamel and cementum – along the entire dentinal tubule, all the way to the most peripheral parts of the dentin.

The structural and functional pattern of dentin, which is revealed exclusively by the SEM method,



a



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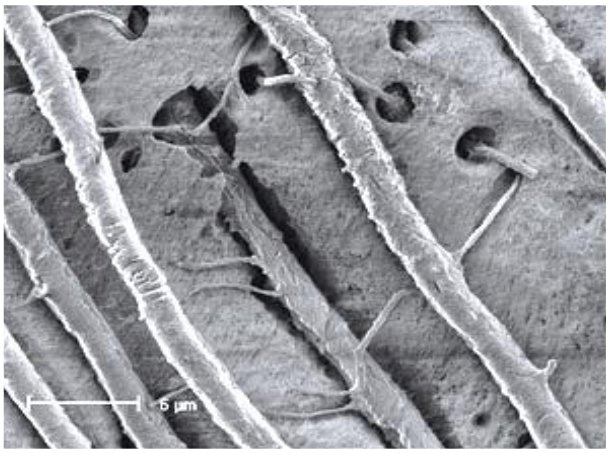


c

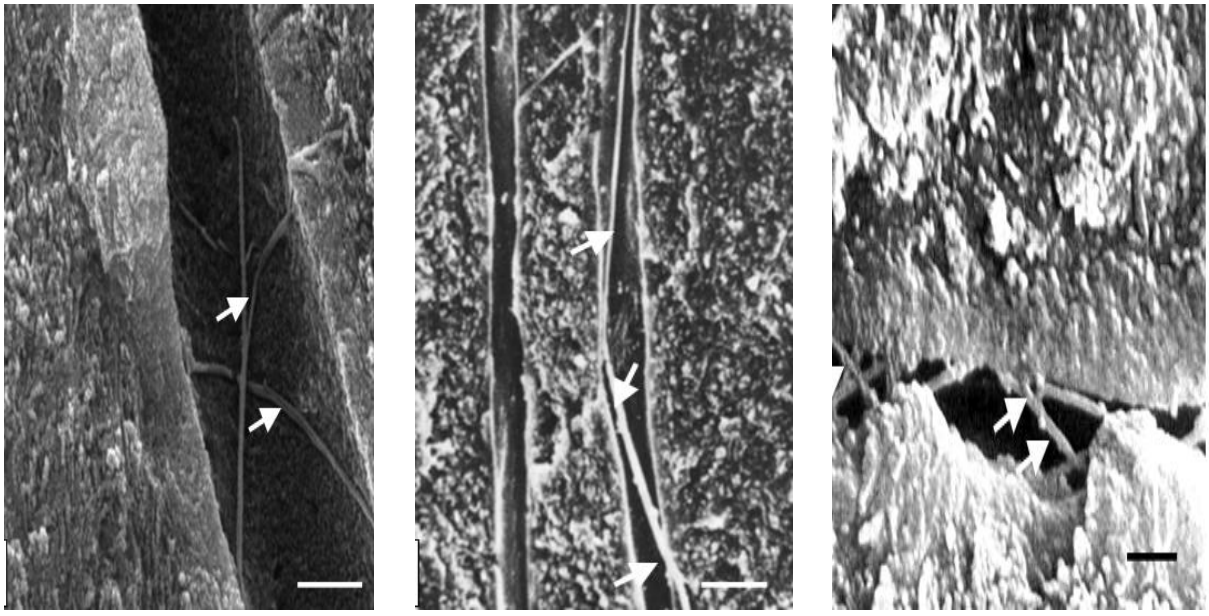
Figure 5: a – general view of the terminal sections of dentinal fibers (1), their fibrillar apparatus (black arrows), lamina limitans biofilm (white arrows); b – general view of the enamel-dentin junction: dentinal tubules (1) with remnants of dentinal fibers (2), terminal thickenings of the DFs (3), which are in contact with the basal layer of enamel (4); lamina limitans (white arrow) is in contact with a similar structure of the adjacent dentinal fiber (black arrow); c – anchor fibers penetrate the lateral tubules without contacting the adjacent DTs. Method: SEM. Accelerating voltage 15 kV; original magnification: a, b × 2500, c × 3000; scale bar: a, b = 10 µm; c = 5 µm

Figure 6 – General appearance of dentin on a slice of a human tooth: 1 – zone of dense secondary dentin (dark stripe); 2 – zone of dentinal tubules with dentinal fibers of normal structure (gray stripe); 3 – zone of dentinal tubules with atrophic dentinal fibers (light stripe); 4 – nodular zone (black stripe). Method: light microscopy (oc. × 7, ob. × 40), impregnation with silver nitrate solution

correlates well with the morphological picture obtained at the light-optical level (Fig. 7). SEM illustrations demonstrate dentinal tubules with multiple microfibrils with an odontoblastic process (Fig. 7a), dentinal tubules with tangentially oriented microfibrils (Fig. 7b), and the presence of interfibrillar microspaces as a morphological substrate for capillary tension forces (Fig. 7c). The zone of dentinal tubules, in which atrophic processes of odontoblasts with reduced microfibrillar material are detected, defines a light stripe of dentin, to which a thin (20-30 μm) nodular zone of "black pearls" (black stripe) adjoins from the outside. The dentinal fibers in the outer ¾ of the length of the DTs disappear, and the DFs themselves often intertwine and bend, which creates the illusion of "pearls" at the photo-optical level.

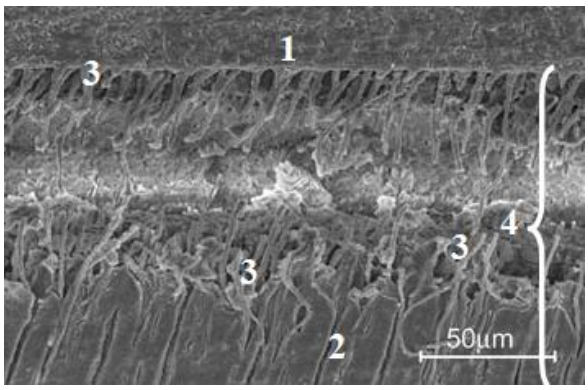
The capillary tension forces that are created between the walls of the DTs and DFs due to the microspaces of the dense network of collagen fibrils are the driving force that ensures the constant circulation of pulp lymph. Such circulation is the basis of living matter, which not only does not contradict the classical model of structural-functional relationships, but also organically substantiates it with morphological research methods, in particular, the SEM method.

A feature of the DFs of the more external dentinal areas was that almost all of them had no microfibrils attached to their surface, or very few of them were visualized. Interestingly, in some areas in the middle third of the dentin, we were able to observe individual DFs that had a smooth wall with a flat or round end at the apex – a club-shaped expansion. The terminal sections of the DFs were suspended on tangentially stretched collagen fibers (Fig. 8).



a b c

Figure 7 – General view of the outer third of dentin: a – dentinal tubules contain microfibrils with a dentinal fiber (arrows); b – dentinal tubules with tangentially oriented microfibrils (arrows); c – interfibrillar microspace. Method: SEM. Accelerating voltage 25 kV; original magnification: a × 2000, b × 1500, c × 10000; scale bar: a = 10 μm, b = 2 μm; c = 1 μm



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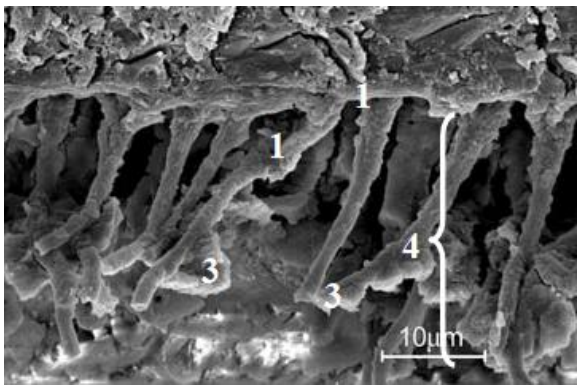

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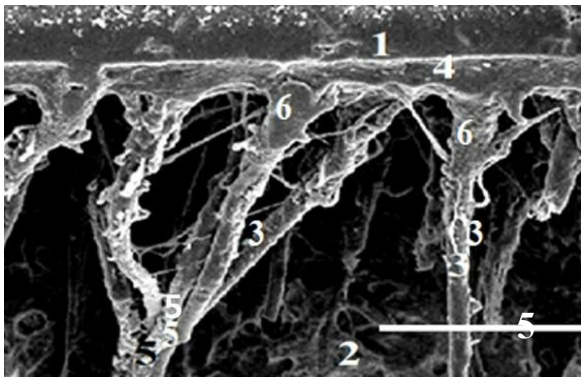

c

Figure 8 – SEM illustration of a black dentin stripe: 1 – enamel; 2 – dentin; 3 – free dentinal fibers; 4 – zone corresponding to the black stripe of dentin; 5 – plexus of dentinal fibers, which creates an illusory image of black pearls under light-optical microscopy; 6 – club-shaped expansions of the dentinal fiber. Method: SEM. Accelerating voltage 10 kV; original magnification × 2500; scale bar: a = 50 μm; b = 10 μm; c = 20 μm

The terminal sections of the dentinal fiber are shown in micrographs (Fig. 9), where microfibrils are visualized in the terminal part of the DFs. It is noteworthy that the end of one dentinal fiber is rounded, while the end of the other two has the appearance of a “broken branch” (Fig. 9a). At the terminal parts of the DFs, individual short microfibrils are found attached only to their surface, and in several cases, microfibrils were attached to the tip of the DF (Fig. 9b). On the walls of the dentinal tubule, devoid of microfibrils, the openings of the anchor microtubules and the anchor tubules themselves are visualized, which blindly end in the peritubular dentin (Fig. 9c). When it is possible to chip the DFs and fix it in an isolated form, short anchor microfibrils can be observed on the surface of the dentinal fiber (Fig. 9d, 9e, 9f). At the same time, it was possible to show blind anchor channel holes located in the walls of the DTs, intended for anchor microfibrils. This creates a gap-like microspace along the entire DT between it and the wall, while the microfibrillar network itself is a source of hydrophilic proteins capable of generating capillary tension forces.

In addition, we observed nodular, smooth-surfaced structures of varying sizes at the enamel-dentine junction. These structures were very common and appeared as isolated or conglomerate nodules, which on longitudinal slices of teeth at the light-optical level define a black stripe (see Fig. 6). Sometimes the structures looked like more or less twisted structures in the form of small knots arranged in a row, with which the DFs ended outside the final segment of the DTs (Fig. 10).

Therefore, modern studies using scanning electron microscopy have become a valuable tool for analyzing the microstructure of dentin and border structures [9]. In the modern sense, dentin morphology studies reveal several dentin-forming microstructures. At the same time, DFs are a basic and extremely complex biological structure, determined by cellular polarization, which reflects the general biological pattern of the development of living matter [31]. Dentinal tubules are described as branchings of dentinal fibers and DF bifurcating lateral branches. At the same time, we [32] found that DFs are associated with openings and windows in the DT walls, which continue into the peritubular dentin and even create a network of contacts with the lateral branches of neighboring DFs in the intertubular dentin. The results of our studies are consistent with the data of Garcés-Ortíz M. et al. [24] on the presence of microfibrillar structures in the dentinal tubules of human teeth, which connect the dentinal tubules and odontoblastic processes. These microfibrillar structures are apparently a support system that serves to support and maintain the odontoblastic process in the dentinal tubule.

In addition, it is assumed that odontoblasts can act as nociceptors – sensory receptors that detect potential danger, particularly exogenous pathogens. Therefore, the study of regeneration processes in the dentin-pulp

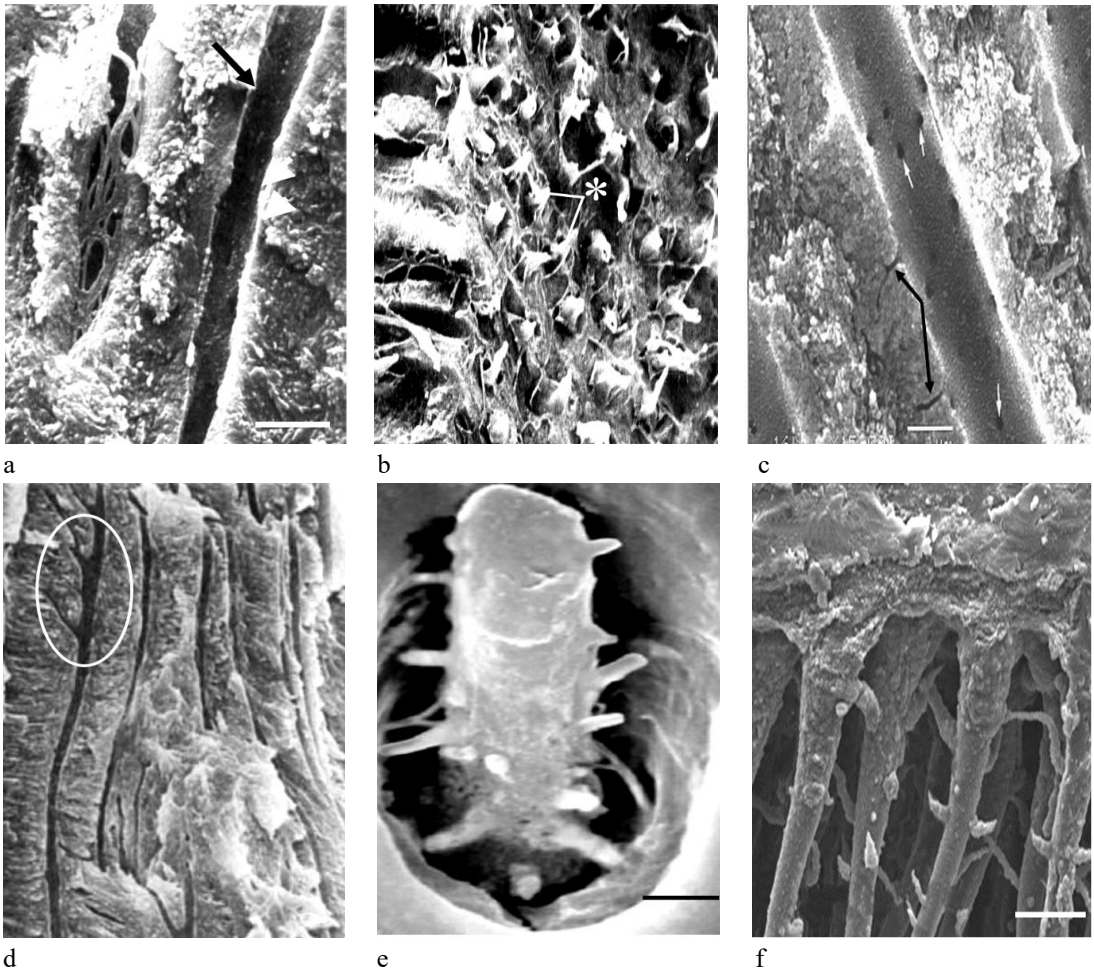

Figure 9 – Terminal sections of the dentinal fiber: a – microfibrils (wide arrows) in the terminal part of the DF, the end of which is rounded (white arrow) and in the form of a "broken branch" (black arrow); b – microfibrils attached to the terminal part of the DF (asterisk); c – walls of the dentinal tubule devoid of microfibrils, anchor tubules blindly end in the peritubular dentin (black arrows), openings of anchor microtubules (white arrows); d – walls of the DTs in a dark stripe of dentin with blind anchor tubules (in a white oval); e, f – short anchor fibrils on the wall of the DFs (in a light stripe of dentin). Method: SEM. Accelerating voltage 25 kV; original magnification: a, b × 5000; c, d, e, f ×1200; scale bar: a, b, g = 5 μm; c, d = 1 μm; f = 2 μm

complex of the tooth remains relevant [33]. The study of dentin morphology and function requires a comprehensive approach to understand the characteristics of vital and non-vital dentin, to model and optimize dentin bonding, and to improve many aspects of preventive and restorative dentistry.

Studying the peritubular and intertubular morphology of dentin, odontoblasts, and their DFs and DTs allows us to predict the presence of a specific structural pattern that serves as a functional and structural unit of dentin in its physiological state. The complex of the structural and functional pattern of dentin, which is well visualized at the light-optical level and during SEM examination, is defined by four dentin stripes: black, dark, gray, and light. Their intensity and width depend on the presence or absence of two other constituent elements of the dentin structural and functional pattern in the dentinal tubule.

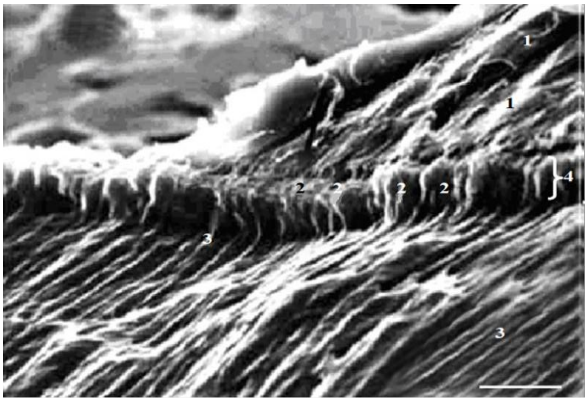

Figure 10 – General appearance of the terminal sections of dentinal fibers in the form of nodular pearls of a black dentinal stripe: 1 – enamel; 2 – nodular expansions; 3 – dentinal fiber; 4 – black dentinal stripe. Method: SEM. Accelerating voltage 25 kV; original magnification × 1500; scale bar = 10 μm

CONCLUSIONS

1. The material presented by us, based on the study of dentin morphology, demonstrates the existence of a certain structural pattern, the components of which are dentinal tubules, odontoblastic processes, and the fibrillar apparatus. They act as a structural and functional unit of dentin in human teeth and are consistently detected regardless of their distance from the pulp chamber.

2. The development of the fibrillar component of this pattern determines the presence of separate stripes of dentin (black, dark, gray, light), which are detected during morphological examination. The central position of the dentinal fiber in each dentinal tubule is ensured by its fibrillar apparatus. The microfibrillar network reflects the structural and functional principles of building living structures, ensures the generation of capillary tension forces, and, accordingly, provides the conditions for the transport of pulp fluid to the enamel, as well as maintaining the stability of the structure and sufficient physiological mineralization of dentin.

3. Our research suggests that studying the complex hierarchical organization of dentin and determining the fullness of its structural and functional pattern will contribute to the development of dental materials with special emphasis on their complex interaction with the dentin matrix and will provide an opportunity to influence the therapeutic approach for treating hard dental tissues.

PROSPECTS FOR FUTURE RESEARCH

Based on the results of our research, expanding the range of knowledge about microstructural changes in tooth dentin during carious lesions is warranted.

ETHICAL CONSIDERATIONS

The studies were approved by the Bioethics Commission of the I. Ya. Horbachevsky Ternopil National Medical University (protocol No. 75 dated 01.11.2023) and were conducted in accordance with the bioethical principles set out in the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Declaration of Helsinki of the World Health Association on Ethical Principles of Medical Research Involving Human Subjects (1964–2000), and the Universal Declaration on Bioethics and Human Rights (UNESCO). Written informed consent was obtained from all the participants.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the development of the initial and revised versions of this article. They are fully responsible for all aspects of the work and for resolving issues related to the accuracy or integrity of the information provided.

Sergii Popel: developed the concept and design of the work, critically reviewed the sections of the manuscript.
Nataliia Gevkaliuk: direct participation in planning, analysis of research results, final approval of the article.
Larysa Tupol: conducting the research process, writing of manuscript sections.
Nataliia Sydliaruk: collection and analysis of research data, writing of manuscript sections.
Vasyl Krupei: writing the initial draft at the pre-publication stage.
Mariana Pynda: critical review, analysis of research results, writing of manuscript sections.

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CONFLICT OF INTEREST

The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

ARTIFICIAL INTELLIGENCE DISCLOSURE

The authors confirm that no artificial intelligence (AI) technologies were used during the writing or editing of the manuscript.

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