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Quantity and diameter of dentinal tubules of human teeth and teeth of experimental animals according to scanning electron microscopy data

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Insight into the interrelation between the structure and mechanical properties of dentin of a tooth is crucial for prevention and treatment of oral cavity diseases. The study of the structure of dental dentin is important for understanding the pathogenetic mechanisms of the spread of cariogenic microflora in progressive dental caries to the pulp and periodontium, as well as for the interpretation of the results of studies of dental dentin-adhesive materials. The in vitro research presented here involved the examination of coronal dentin in 46 intact teeth from rats, cats, dogs, and humans at various distances from the pulp chamber, with a focus on the quantity and diameter of dentinal tubules. The calculations were based on the analysis of 155 scanning electron microscope photographs of the enamel surface and pulp chamber wall. The researchers calculated the quantity and diameter of dentinal tubules per unit area, which they measured at various distances from the pulp chamber. It was found that dentinal tubules are the widest near the pulp chamber wall, where the average diameter ranged from 1.7 to 2.8 µm. The highest values were found in premolars of young humans and the lowest - in the incisors of rats. In the middle portion of the dentin, the diameter of dentinal tubules ranged from 1.0 to 13.3 µm, while at the periphery, it was 0.6–0.9 µm. The highest density of dentinal tubules per unit area was found closest to the pulp horn, and the lowest was in the enamel region. Near the pulp chamber wall, the average density of dentinal tubules ranged from 50 000 to 90 000/mm², in the middle of the dentin, it ranged from 37 000 to 50 000/mm², and at the periphery, it ranged from 10 000 to 25 000/mm². All examined teeth exhibited straight dentinal tubules, except for the rats' incisors, which showed local irregularities. A comparison was made based on the quantity and width of dentinal tubules in the examined animals and humans.

Keywords: microstructure of dentin; dentinal tubules; dentin hypersensitivity; demineralization; animal teeth; human teeth; scanning electron microscopy.

Introduction

Insight into interrelation between the structure and mechanical properties of dentin of teeth is crucial for prevention and treatment of oral cavity diseases. Dental tubules fulfill a substantial role in transmitting stimuli to the dental pulp. This way and through diffusion in the liquid that fills the dentin tubules, in cases of advanced tooth decay with exposed dentin, cariogenic microflora can penetrate into the pulp and periodontium. Inadequate preparation of individual tooth areas may leave infected tissues, which can lead to an exacerbation of the inflammatory process in the pulp and periodontium. Dental pain arises from exposed dentin following bacterial, chemical, or mechanical erosion of enamel or recession of gingiva. Thus, dentin in general and open dental tubules in particular are the first structure involved in dentin sensitivity. Interestingly, the architecture of dentin could facilitate the transfer of information to the underlying dental pulp via odontoblasts (dentin-forming cells), via their apical extension bathed in the dentinal fluid running in the tubules, or via a dense network of trigeminal sensory axons intimately related to odontoblasts (Magloire et al., 2010). Dentin hypersensitivity is a common condition, it afflicts 15–20% of the adult population, typically 20 to 50-year-olds, with peak incidence between 30 and 39 years. Some studies have reported higher prevalence levels of up to 57%. The incidence of dentin hypersensitivity is expected to rise with changing diets, and as caries and periodontal disease prevention result in improved oral health status (Cummins, 2009). Dentin hypersensitivity adversely affects quality of life by impairing the ability to eat and drink certain foods, therefore, should be addressed promptly with a contingency plan that includes initial treatment with a noninvasive prophylactic home care approach, followed by in-office treatment if desired result is not achieved. For today, many studies have been conducted to assess the decrease in dentin permeability and the presence of nanoleakage in the resin-dentin complex when using universal adhesives (Midha et al., 2021). It was found that no adhesive system completely reduced dentin permeability. Various therapeutic agents applied topically to exposed dentin can also reach the pulp, and the rate of delivery of therapeutic agents to the tooth pulp depends on the quantity and size of the dentinal tubules. Nowadays researchers' attention is focused on studying the microstructure of dentin, as comparative characteristics of the density and diameter of dentinal tubules in the crown portion of the tooth are important for investigations of dentin adhesive materials. The scientific research task of deepening knowledge about dentin tissue is aimed at combining information about its microstructure and its associated mechanical properties. Experimental studies on the pulp reaction after the application of various agents to dentin are often conducted on animals. However, information regarding the quantity and diameter of dentinal tubules in experimental animals is contradictory. Studies assessing dentinal tubules in humans and other mammals have shown significant spatial and morphological variations both within and between individuals and species. Soukup et al. (2023) examined microstructural features of dentin in the maxillary molars of mature dogs and investigated quantitative relationships between mechanical properties and the complex microstructure of dogs' dentin. As far as it is known, data on the dentin morphology of humans and other mammals, prepared in the same way for in vitro microstructure studies, are limited and often contradictory. Therefore, our task was to determine the quantity and diameter of dentinal tubules in standardized preparations of human dentin and dentin from other mammals and to conduct a comparative evaluation.

The objective of this study is to investigate the quantity and diameter of dentinal tubules in the pulp chamber wall and coronal dentin of teeth in rats, dogs and humans of different ages, and to carry out a comparative analysis.

Materials and methods

The animals were used in the experiment in compliance with the principles of bioethics in accordance with the legislation of Ukraine (Law of Ukraine No. 3447-IV, 2006), the rules of the European Convention on the Protection of Vertebrates animals used for experimental and other scientific purposes (Council of Europe Directive 86/609/EC, 1986), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment", general ethical principles of animal experiments, adopted by the First National Congress of Bioethics of Ukraine (2001). The study design was approved by the Commission of Bioethics of Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 59 dated June 5, 2020). Written informed consent for participation in the study was obtained from the patients.Violations of moral and ethical norms during the conduct of research work were not detected.

We examined a total of 27 teeth of sexually mature animals, including permanent incisors and molars from rats, permanent canines from cats and dogs, as well as 19 permanent incisors and premolars from the upper jaw of humans aged 12 and 65 years. Tooth extractions in experimental animals were performed under thiopental anesthesia. Tooth extractions in individuals were carried out for clinical indicators under local anesthesia. The teeth were placed in 10% neutral formalin, after which dental sections were made (Fig. 1).

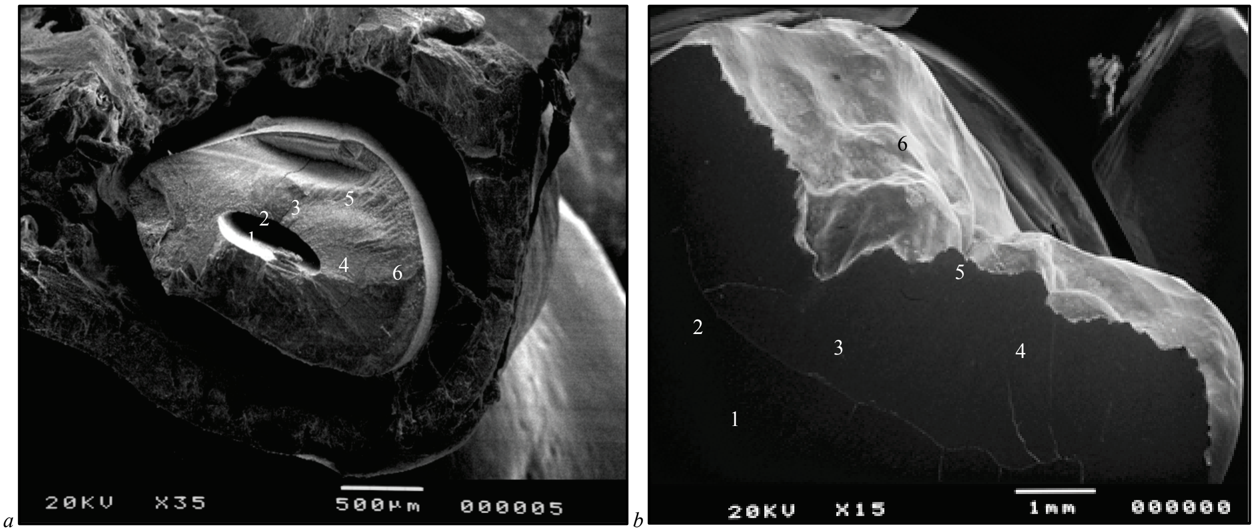

Fig. 1. Transverse section of the canine tooth (*a*) and longitudinal section of the premolar tooth (*b*): *1* – pulp chamber; *2* – directly adjacent to the pulp; *3* – 50–100 µm away from the pulp or at half the thickness of the dentin; *4* – dentin; *5* – at the enamel-dentin junction; *6* – enamel; scanning electron microscopy; accelerating voltage 20 kV

After the sectioning, the teeth were treated with a 3% hydrogen peroxide solution, rinsed in running water, and placed in a 10 % solution of neutral formalin. Prior to examination using scanning electron microscopy (SEM) in a vacuum metal deposition instrument "VUP-5M" (SELMI LLC, Sumy, Ukraine), the fracture surface was coated with a layer of chemically pure aluminum (99.9% purity) with the thickness of approximately 200 A0. For the investigation, a scanning microscope "JEOL-25M-T220A" (Japan) with an accelerating voltage of 20 kV was used. A total of 155 images were taken at various distances from the pulp chamber (Fig. 1a). The diameter of dentinal tubules was measured on photographs of cross-sections using a program developed by Garberoglio & Brännström (1972), which allows measurements with an accuracy of 0.1 mm. The average diameter of 8–20 tubules was calculated from each reviewed photograph. To minimize measurement errors that may occur when using low magnification in SEM, diameter calculations were based on sample magnifications exceeding 2000 times.

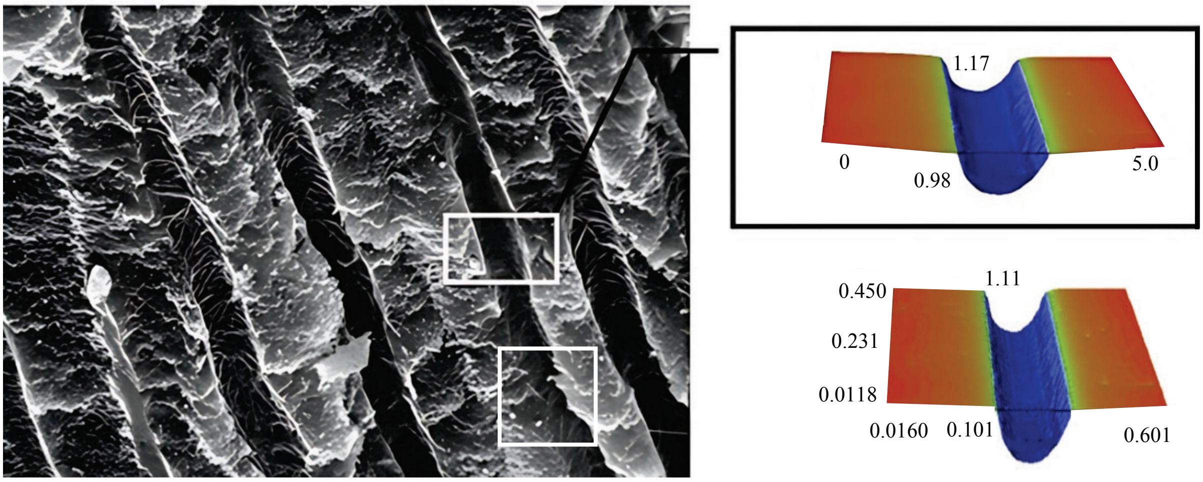

Fig. 2. The examples of studies on the morphometric parameters of dentinal tubules in photographs taken with scanning electron microscopy: to assess the overall structural arrangement of dentinal tubules, tooth sections were prepared, and they were impregnated with a 3% silver nitrate solution (Fig. 3)

Images of cross-sectioned dentinal tubules were also used to calculate their quantity per 1 mm². If the magnification revealed a large number of dentinal tubules, the calculation was facilitated by overlaying a measuring grid on the image, which allowed marking the tubules for their counting. Knowing the magnification, the area of the photograph was estimated in mm², and the number of dentinal tubules in that area was calculated using by Absi EG methodic (Absi, 1987). The percentage of the area occupied by dentinal tubules was calculated by multiplying the number of dentine tubules per mm² by the radius of the tubules in micrometers and dividing by 100. The program allows automatic determination of dentinal tubule diameter in any zone along their entire length (Fig. 2, 3). Statistical analysis of the data was performed using parametric methods. The difference between the mean values was considered significant at $P < 0.05$.

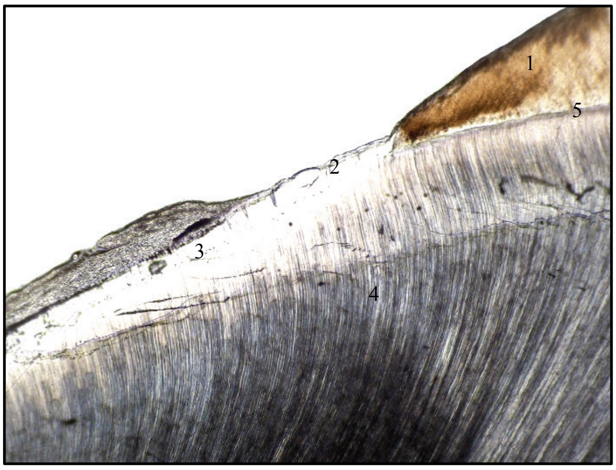

Fig. 3. The general structure of dentinal tubules: *1* – enamel, *2* – tooth neck, *3* – root cementum, *4* – dentin, *5* – enamel-dentin junction; tooth section prepared and impregnated with a 30% silver nitrate solution; magnification: ×600

Results

In all the examined teeth, the largest diameter of dentinal tubules was found near the wall of the pulp chamber. In this area the average diameter ranged from 1.6 to 2.7 micrometers, with the highest values of dentinal tubule diameter observed in the incisors of young humans (Fig.4), and the lowest values observed in the incisors of rats (Fig. 5).

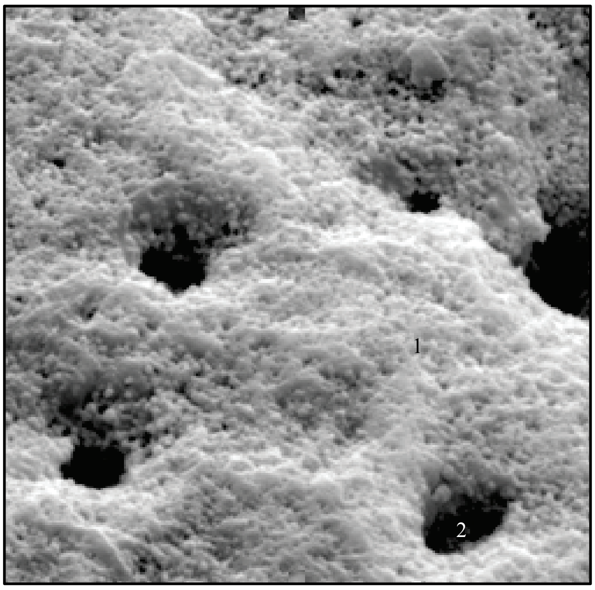

Fig. 4. The structure of dentin in a 12-year-old human incisor: *1* – dentin, *2* – dentinal tubule; the average diameter is 2.7 µm; scanning electron microscopy, accelerating voltage 20 kV; magnification × 3350

The wall of the pulp chamber at the crown level of the tooth, where the membranous structure is similar to that presented in Figure 4, but was removed by treatment by a 10% sodium hypochlorite solution. The number of dentinal tubules before treatment with sodium hypochlorite solution was 69,000/mm² (Fig. 5).

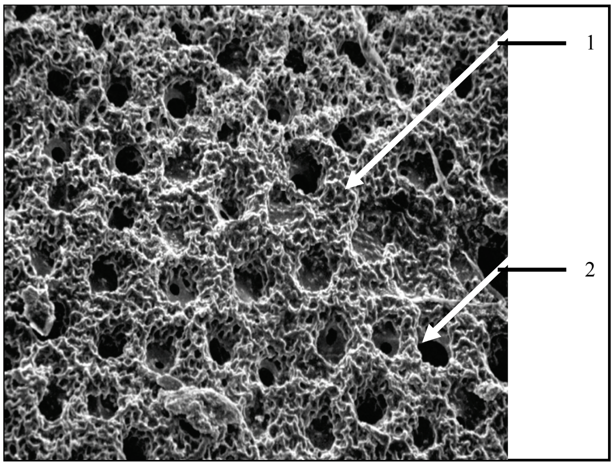

Fig. 5. The structure of dentin in the wall of the pulp chamber of a rat incisor: *1* – dentin, *2* – dentinal tubule; the walls are covered with a membranous structure that contains a fibrous, finely porous component; the number of tubules: 73 000/mm²; average diameter: 1.4 µm; magnification × 3350

The width of dentinal tubules noticeably decreased at a distance of 50–100 µm from the pulp chamber, which corresponds to the level of development of peritubular dentin. From this level, the diameter gradually decreases towards the enamel-dentin junction. At the midpoint between the pulp chamber and the enamel, the average diameter of dentinal tubules ranged from 1.1 to 1.4 µm, and near the enamel, it varied from 0.6 to 0.8 µm (Fig. 6).

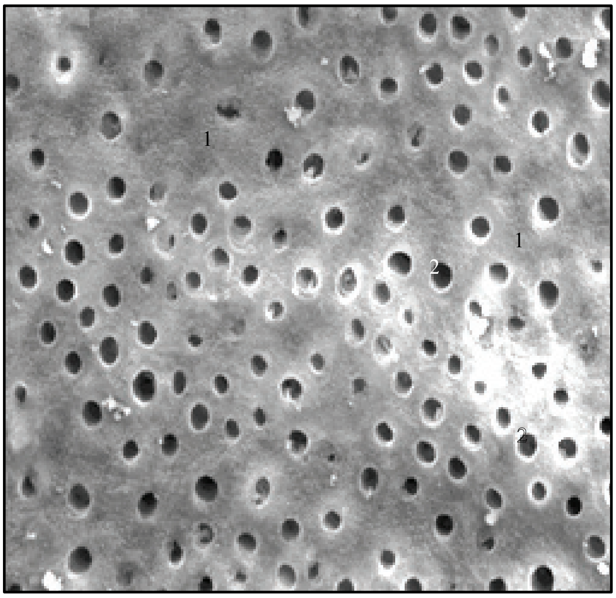

Fig. 6. The structure of dentin in a cat's molar: *1* – dentin, *2* – dentinal tubule; the cervical third of the tooth crown, approximately 100 µm from the pulp chamber wall, shows irregularities in the width of dentinal tubules; the number of tubules was 58 000/mm², with an average diameter of 1.2 µm; magnification ×4 600

In all samples, a very high variability in the diameter of dentinal tubules was observed, which is well visible on the wall of the pulp chamber (Fig. 7). Dentinal tubules located closer to the coronal part of the dentin had a smaller average diameter compared to those in the cervical part and exhibited greater mutual variability, especially in areas near the pulp chamber. Further away from the walls of the pulp chamber, the diameter of dentinal tubules in the teeth of experimental animals was more stable (Fig. 8).

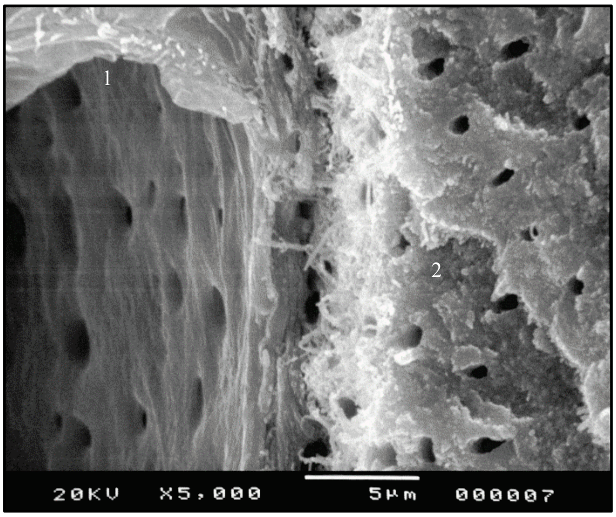

Fig. 7. The structure of dentinal tubules in a cat's molar from the side of the pulp chamber wall: *1* – pulp horn; *2* – parapulpal dentin; the number of dentinal tubules 45 000/mm², with an average diameter of 0.6 μm; scanning electron microscopy; accelerating voltage 20 kV

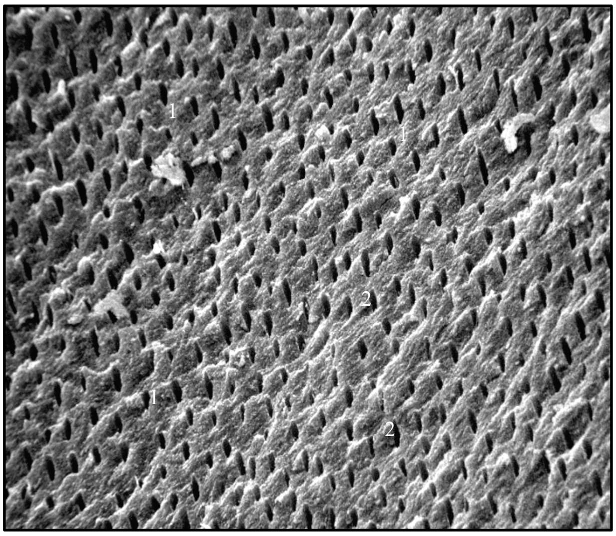

Fig. 8. The structure of dentin in the crown of a dog's molar 1.7 mm away from the pulp chamber wall (the total distance from pulp to enamel is 1.8 mm); *1* – dentin, *2* – dentinal tubule; the number of tubules is 76 000/mm², with an average diameter of 1.0 μm; magnification ×1830

In this case, the structure of dentin in a dog's incisors is not different from that observed in the dentin of teeth in other animal species (Fig. 9).

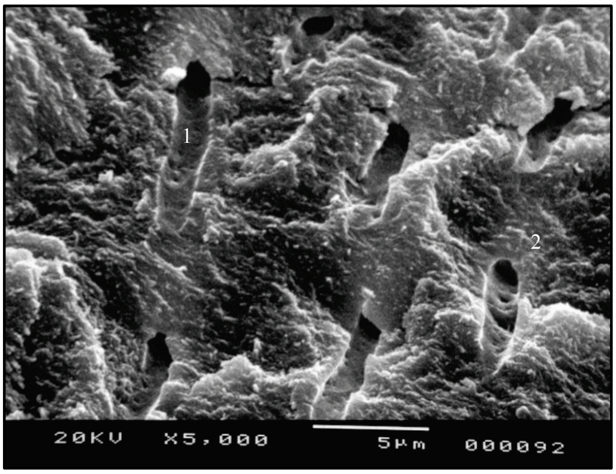

Fig. 9. The structure of dentin in the crown of a dog's incisor near the dentin-enamel junction; *1* – dentin, *2* – dentinal tubule; the number of dentinal tubules is approximately 24 000/mm², accelerating voltage is 20 Kv; magnification ×5 000

The diameter of dentinal tubules farther away from the wall of the pulp chamber was not stable in the dentin of rat incisors, where branching with lateral extensions into the intertubular dentin was observed. These were typically distorted dentinal tubules with lateral branching structures (Fig. 10).

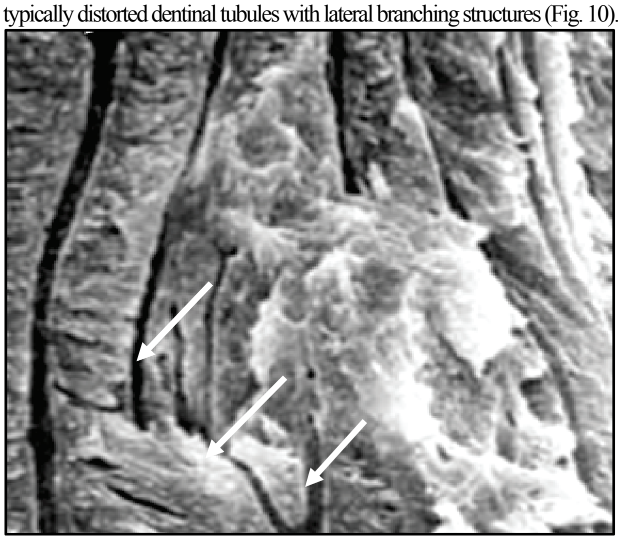

Fig. 10. The structure of dentin in a rat's incisor near the enamel-dentin junction of the crown: longitudinally sectioned dentinal tubules with lateral branching structures (arrows) can be observed; magnification ×1150

The average diameter of dentinal tubules varies at different distances from the pulp chamber, as indicated in Table 1.

Table 1
Diameter of dentinal tubules (μm) at various distances from the pulp chamber of the examined teeth

Distance from the pulp chamber	Number of dentinal tubules, μm				human premolar aged 12 (n = 7)
	rat incisor (n = 35)	rat molar (n = 35)	cat molar (n = 35)	dog molar (n = 35)	
Pulp chamber wall	1.712 ± 0.032^a	2.131 ± 0.043^b	2.114 ± 0.031^b	2.132 ± 0.032^b	2.842 ± 0.033^c
50–100 μm from the pulp chamber	1.133 ± 0.011^a	1.323 ± 0.022^b	1.212 ± 0.021^b	1.923 ± 0.022^c	1.732 ± 0.021^c
Tissue in between the pulp chamber and enamel	1.021 ± 0.011^a	1.143 ± 0.011^b	1.021 ± 0.012^a	1.313 ± 0.012^c	1.123 ± 0.012^b
Internal dentin-enamel junction	0.723 ± 0.032^a	0.814 ± 0.021^b	0.732 ± 0.002^a	0.914 ± 0.005^c	0.922 ± 0.003^c

Note: different letters in a row indicate that data samplings are significantly ($P < 0.05$) different one from another according to the Tukey test with the Bonferroni correction.

The highest number of dentinal tubules per unit area was found in the dentin of the tooth crown. This number was slightly lower than in the part near the pulp chamber wall and the dentin in the middle part of the crown. The lowest number of the dentinal tubules was observed in the cervical part of the incisor crowns. In all examined teeth, the number of dentinal tubules was the lowest near the enamel-dentin junction (Fig. 11). The quantity of dentinal tubules per unit area at various distances from the pulp chamber of the examined teeth is provided in Table 2. It was of interest to compare the structure of non-decalcified dentin in human teeth with the values we obtained from experimental animals. Table 3 provides the average values of the diameter, the number of dentinal tubules per unit area, and the percentage of the area occupied by the dentinal tubules at half the distance between the pulp chamber and the enamel in human dentin and in the examined animals.

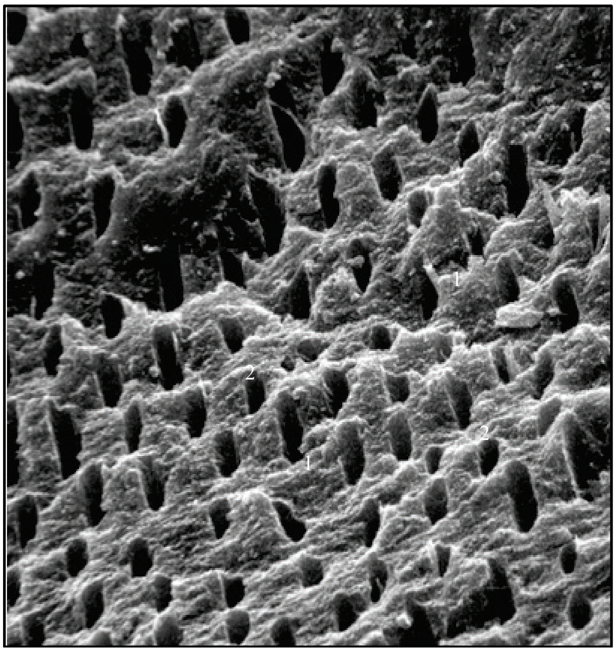

Fig. 11. The structure of dentin in a rat's molar near the enamel-dentin junction: *1* – dentin, *2* – dentinal tubule; the number of dentinal tubules is 50,000/mm²; average diameter of 1.2 µm; magnification ×1575

The highest variation in dentinal tubule diameter and the greatest variability was observed near the pulp chamber wall or within the predentinal zone. At half the distance from the pulp chamber to the enamel of the tooth, the average number of dentinal tubules varied from 37 000 to 50 000/mm², while in the part close to the enamel, this number ranged from 10 000 to 25 000/mm² (Table 2). The dentinal tubules in the molars of rats, cats and dogs have a relatively straight orientation, but in the incisors of rats, they appear to be locally curved (Fig. 12).

Table 2
Number of dentinal tubules at various distances from the pulp chamber of the examined teeth (K/mm²)

Distance from the pulp chamber	Number of dentinal tubules				
	rat incisor (n = 35)	rat molar (n = 35)	cat molar (n = 35)	dog molar (n = 35)	human premolar aged 65 (n = 8)
Pulp chamber wall	51 ± 2	64 ± 3	50 ± 3	90 ± 4	51 ± 3
50–100 µm from the pulp chamber	53 ± 2	80 ± 4	59 ± 3	74 ± 4	47 ± 3
Tissue in between the pulp chamber and enamel	50 ± 2	49 ± 4	41 ± 2	43 ± 3	37 ± 2
Internal dentin-enamel junction	16 ± 1	25 ± 2	25 ± 2	24 ± 1	10 ± 1

Table 3
The average values of dentinal tubule diameter, the number of tubules/mm², and the percentage of the area occupied by them in the examined teeth

Type of teeth	Diameter of dentinal tubules, µm	Number of tubules/mm²	Percentage of the area/unit of dentin area
Rat incisor (n = 35)	1.0	49 000	3.8
Rat molar (n = 35)	1.1	48 000	4.5
Cat molar (n = 35)	1.0	40 000	3.1
Dog molar (n = 35)	1.2	41 000	5.6
Human premolar (age 12) (n = 7)	1.4	28 000	4.5
Human incisor (age 65) (n = 8)	1.1	27 000	2.7

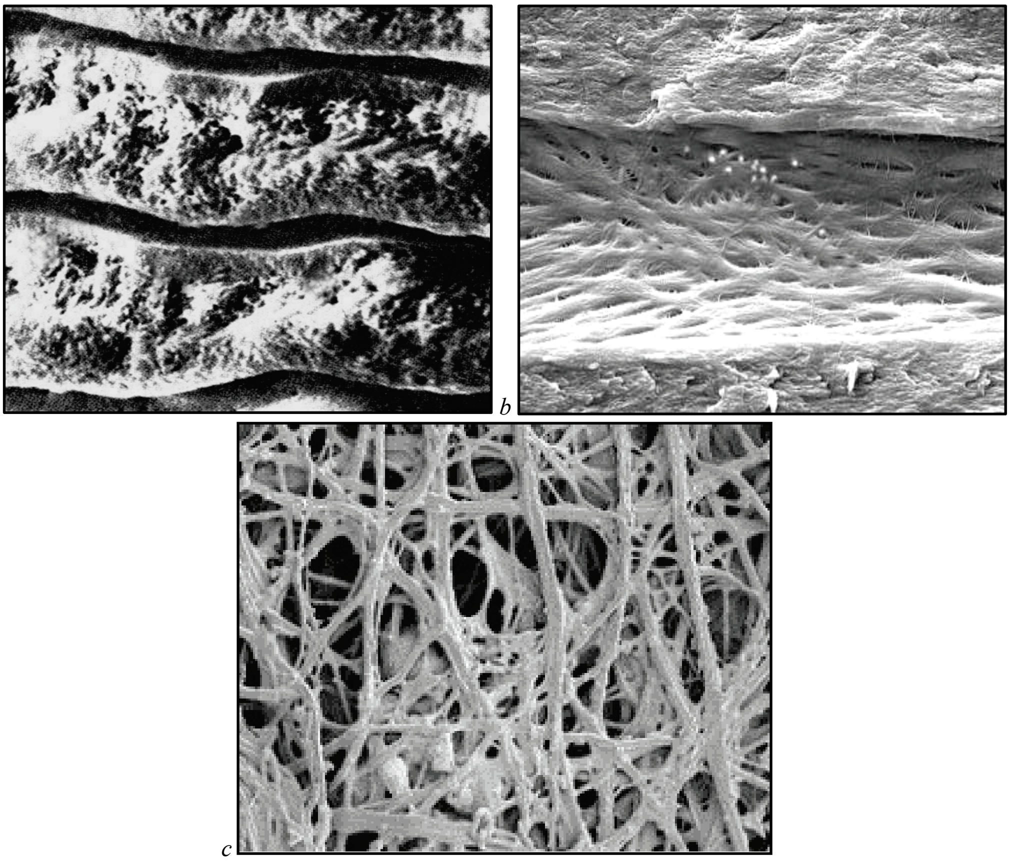

Fig. 12. The character of the dental tubule arrangement in a rat incisor (*a*): the middle part of the crown; longitudinally sectioned dentin (*b*), showing a dense network fibrillar structures on the dentinal tubule walls (*c*); scanning electron microscopy; accelerating voltage 20 kV; magnification ×1280

Certain areas of dentinal tubules lack a fibrillar lining or have only its contours because they are concealed under a thin layer of cementing material. This makes the surface of the dentinal tubule relatively smooth and does not affect its diameter throughout its length. The presence of a dense network of fibrillar structures on the walls of dentinal tubules may serve as a basis for bacteria flora penetration into the dentinal tubules (Fig.13). We propose naming this organic mesh-like structure present on the inner surface of dentinal tubules throughout their length as the "lamina limitans" or "limiting lamina", following the terminology established for bone and mineralized cartilage. We believe that the "lamina limitans" corresponds to the fibrils found in soft tissues, as first described by Thomas & Payne (1983) within the dentinal tubules. Morphometric analysis revealed certain age-related patterns in the distribution of dentinal tubules depending on their topography in the wall of the pulp chamber of human teeth (Fig.14). The histogram depicting the distribution of various dentinal tubule parameters indicates a clear age-related pattern of decrease in each parameter in humans. This information is essential for consideration in the practice of a dentist.

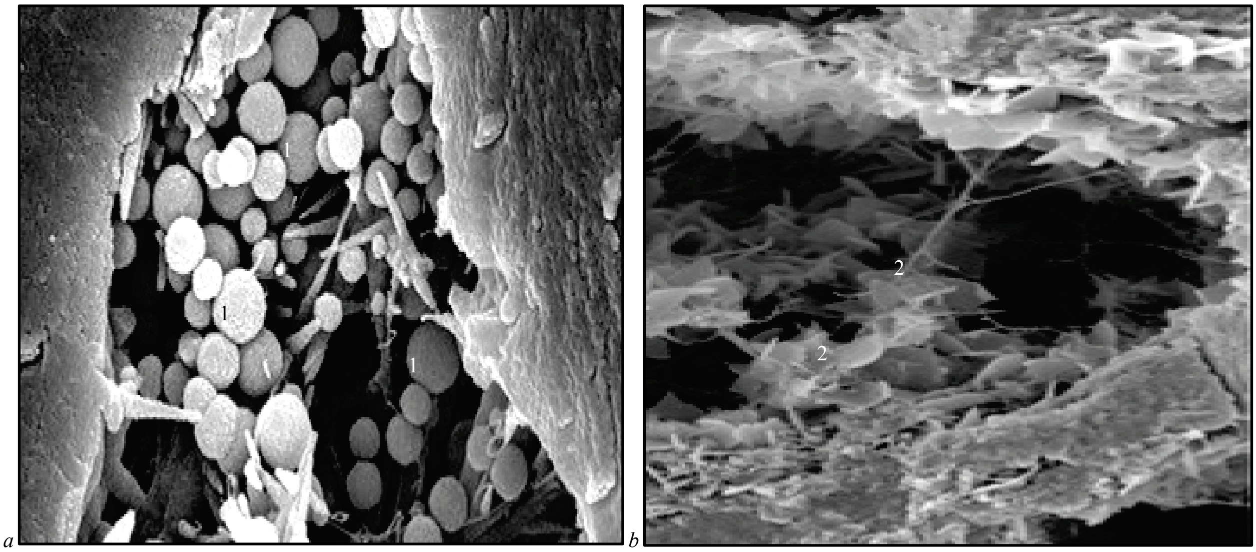

Fig. 13. The coccus flora (*a*) within the dentinal tubule, with the dense fibrillar mesh-like structure (*b*): *1* – coccus flora, *2* – fibrillar mesh-like structure; scanning electron microscopy; accelerating voltage 20 kV; magnification ×1500

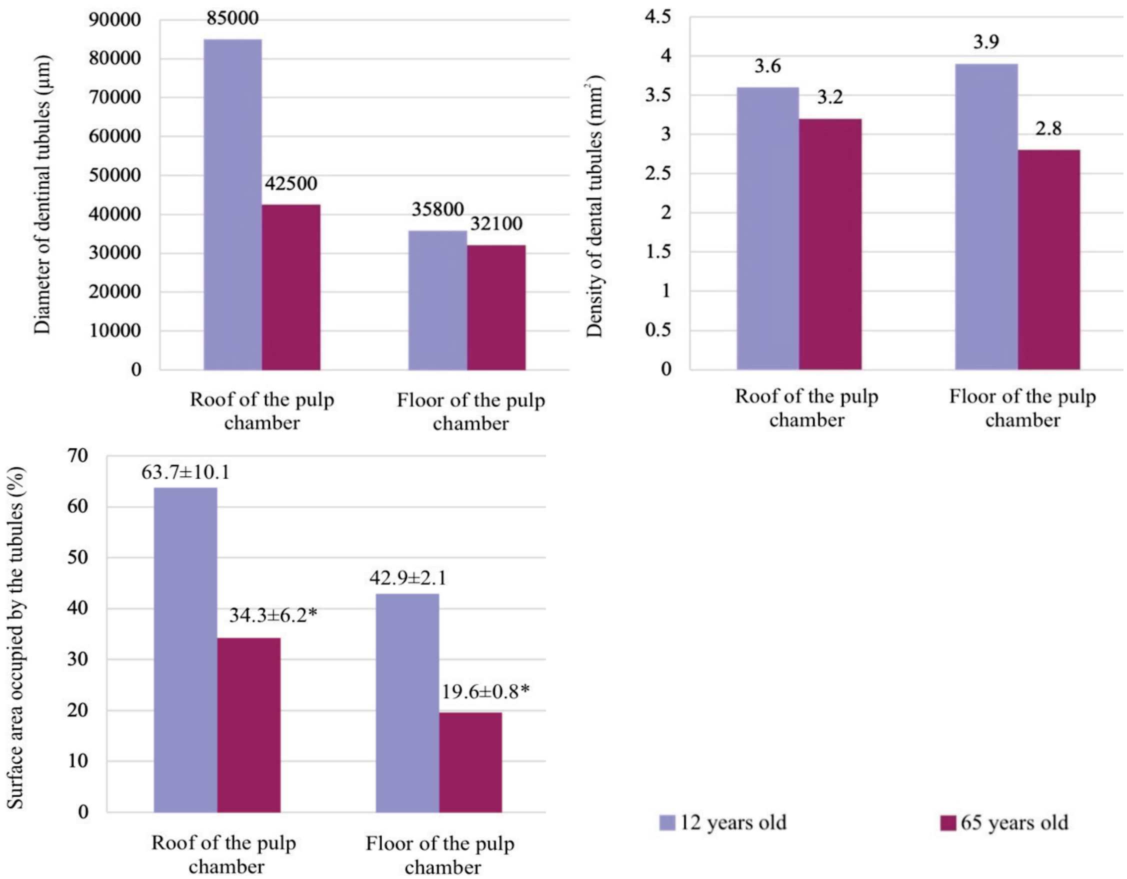

Fig. 14. Morphometric parameters (mean values ± standard deviations) of dentinal tubules depending on their topography of the pulp chamber in individuals of different ages: * – P < 0.05

Discussion

The data obtained from measurements of this type are susceptible to certain errors that can affect the mean values of dentinal tubule counts and their diameters. Some errors, such as the values of magnification and the tilt angle of the specimen, are inherent to the SEM procedure itself, while others are related to difficulties in accurately determining the diameter of dentinal tubules in the photographs. The primary focus of this study was to compare the width and quantity of dentinal tubules in a range of experimental animals and relate the results to similar parameters obtained in humans. Since all specimens were examined in a consistent manner, errors inherent to the methodology were effectively canceled out in relation to each other. In order to obtain reliable information about dentinal tubules, it is important for the research to be conducted on dentin that has not been decalcified. When studying decalcified human dentin, researchers found that the diameters of dentinal tubules were larger than those reported for non-decalcified dentin (Fromme & Riedel, 1970; Garberoglio & Brännström, 1972; Pessoa et al., 2013; Garcés-Ortíz et al., 2015; Kranz et al., 2022). Clearly, one of the reasons for this discrepancy is that the decalcification leads to the almost complete removal of peritubular dentin. It is not surprising that the width of the tubules varies on the pulp-facing wall lined with pre-dentin. Cytoplasmic structures and membranes can even be preserved in sublimated samples (Fromme & Riedel, 1970). During fixation with formalin solution, samples are dehydrated in a vacuum, and these structures seem to adhere to the wall of the pulp chamber. Therefore, it was sometimes difficult to assess the diameter of dentinal tubules. When most of the pre-dentin was removed using sodium hypochlorite, the width of dentinal tubules was generally larger and uniform. Thus, standardizing the measurement methodology should provide a more reliable assessment of dentinal tubule diameter in this area (Fig. 2). With this in mind, we propose using a standardized method for measuring dentinal tubule dimensions to avoid the current wide divergence of opinions and research results that exist in the scientific literature today.

Additionally, the organic material covering the pulp chamber wall interferes with the counting of tubules, as some tubules were completely covered by fibrillar structures as shown in Figure 11. The data on human dentin presented in Table 3 were provided based on the information obtained from the study by Garberoglio & Brännström (1972), which is available only as an appendix to a dissertation research in which essentially the same method as used in this study was employed. It is evident that the value of data on dentin from various animal species lies in the fact that structurally, it does not significantly differ from human dentin data. Only dentin in rat incisors showed irregularities and branching to a degree not observed in human dentin or in other species we investigated. When isolated branching did occur in human dentin, it was confined to the area of the enamel-dentin junction (Fromme & Riedel, 1970; Riedel, 1970; Brännström & Johnson, 1974; Pessoa et al., 2013; Mahdee et al., 2016) and was more of a sporadic exception.

The results of this study have shown that the diameter and quantity of dentinal tubules in animals and humans are similar. It is known from research findings that the delivery of different substances and the bulk flow of fluids in dentin depend on the presence of open dentinal tubules. Therefore, the permeability of dentin, which is dependent on the state of dentinal tubules, may be similar in rats, cats, dogs and humans. This justifies the position that the results obtained in animals can be interpreted for comparative characterization of these parameters in humans. Thus, physiological and histological research results on dentin permeability and pulp responses conducted *in vitro* on animals can also be representative for human dentin and pulp.

Conclusions

Dentinal tubules in different teeth exhibit a fundamentally uniform structural organization, and with age, the number of tubules remains unchanged, as evidenced by the nearly consistent density of their distribution within an area of 1 mm². Dentinal tubules in rat incisors exhibit local curvatures and lateral branching. The lowest number of dentin tubules is found near the enamel-dentin junction.The highest percentage of area per unit of dental area is observed in dog molars and amounts to 5.6, whereas the lowest is determined in elderly humans. The horn of the pulp chamber has larger dentin canal diameters and a greater area of canal exposure compared to the floor of the pulp chamber, where canal diameters are smaller, especially in young individuals. With age, the density of canal placement decreases, the canal diameter decreases and the percentage area of canal openings on the walls of the pulp chamber decreases. The inner walls of dentin canals have numerous fibrillar folds and mesh-like structure that can trap bacterial microflora in the canals when they become infected. The walls of dentin canals are covered by three types of membranous structures: the walls of dentin canals are entirely covered by a membranous structure; the walls of the canals are partially filled with cementing substance that occupies the interfibrillar space; walls that have been completely deprived of membranous structures or cementing substance.

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The authors declare no conflict of interest.

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