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Evaluation of dentinal tubules of dentin of the roots of necrotic teeth by means of scanning electron microscope

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ABSTRACT

Introduction: Dentin is porous due to existence of tubules, containing cell knobs and nerve fibers. It forms integral part of the tooth.
Aim: The aim of the study was measurement of the width of dentinal tubules of the roots of necrotic teeth with chronic periapical inflammation after endodontic treatment as well as differences related to age and gender.
Material and methods: The study comprised 53 teeth (30 molars, 23 premolars) extracted at the Oral Surgery Department, Medical University of Lublin. The teeth were divided into two groups: 40 teeth extracted because of periapical lesions, 15 of which had been treated endodontically before, and the control group of 13 healthy teeth extracted for orthodontic reasons.

Scanning electron microscope was used to evaluate the construction of dentinal tubules. The diameter of dentinal tubules was measured close to the cementum and near the dentinocemental junction.
Results and discussion: The study demonstrated larger diameter of dentinal tubules in necrotic teeth roots compared to the width of tubules of vital teeth. In the control group the mean tubule diameter near the cement was 1.38 μm, and nearby the root canal 3.10 μm. In the group of devitalised teeth the average diameter measured in the same position amounted to 3.68 μm and 4.89 μm.
Conclusions: The width of dentinal tubules of necrotic teeth was significantly higher compared to the teeth with vital pulp. Endodontic treatment does not change the width of dentinal tubules. The width of dentinal tubules does not depend on age and gender.

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1. Introduction

Dentin is a hard, mineralized connective tissue that provides the bulk of the tooth.[1–3] It is located between the chamber filled with dental pulp and tooth enamel at the crown and between the cementum and the pulp of the tooth root. Dentin thickness ranges from 3.0–3.5 mm in the coronal section tapering toward the root apex to 0.8–1.3 mm.[2] Dentin construction resembles bone construction, however, it is much harder. It is characterized by high flexibility and elasticity, which determines its resistance to fracture.[1,2,4]

A characteristic feature of dentin is high porosity due to channels passing through the entire thickness thereof. The diameter of the tubules tapers toward the periphery; tubules adjacent to the pulp have a diameter of 2.0–3.0 μm and taper to about 0.6–0.8 μm close to the dentinocemental junction. Also the number of the tubules changes depending on the distance from the pulp chamber. Close to the chamber there are approximately 48 000 tubules/mm^2, in the middle approximately 37 000 tubules/mm^2, and close to the dentinocemental junction around 22 000/mm^2.[1,4,5]

Dentin–pulp complex and interdependence between the two tissues are claimed to be an integral part of the tooth. Pulp and dentin are embryologically, histologically and functionally the same tissue and therefore are considered as a complex. Both tissues derive from mesenchymal dental papilla (*papilla dentis*). Pulpo-dentin complex is one structure due to pulp cell knobs and nerve fibers placed within dentinal tubules. Dentin sensitivity to stimuli depends on the pulp and pulp reactions depend on changes in dentin. The interdependence of the two tissues is observed in inflammatory processes of the pulp.[1,2,6,7]

Pulp damaging factors include attrition, erosion, abrasion, recession, tooth decay, as well as mechanical, chemical and thermal factors. In case of minor damage to the tooth higher concentration of transforming growth factor β (TGF-β) in the pulp chamber occurs. TGF stimulates the odontoblasts lying around the damaged tissues and therefore an increase in the secretion of building factors leading to the formation of reparative dentin appears. When the pathogen exceeds the ability of the tooth to defend, inflammation of the pulp develops. Inflammatory response begins at the approximation of pathogens at a distance of 0.5 mm from the pulp chamber.[8] Inflammation is accompanied by pH decrease of the interstitial fluid in the pulp and dentinal tubules. Upon destruction of odontoblastic layer, TGF-β stimulates odontoblast precursor cells present in the pulp to proliferation and chemotaxis. The cells take the place of the destroyed odontoblasts and start to accumulate tertiary dentin.[9]

Pulp inflammation in the beginning is limited only to the action of toxic agent, but after 72 h a spread of inflammation throughout the pulp and destruction of the odontoblastic layer appears. In response to the action of bacterial toxins vasoconstriction and increased tissue fluid flow in the outer layers of the dentinal tubules begin. Increased tissue perfusion reduces toxicity and removes bacteria from the tissues. At the same time cellular and immune response are activated. Odontoblasts begin to produce numerous growth factors such as bone morphogenetic protein 2 (BMP-2), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), TGF-β which participate in mineralization of dentin matrix. During pulp inflammation concentration of the inflammatory mediators as histamine, serotonin, and prostaglandins increases. Inflammatory markers have a tendency to diffuse through the tubules into saliva surrounding the tooth.[10]

After vasoconstriction, vasodilation occurs, leading to the accumulation of red blood cells in the central part of blood vessels and leukocyte migration and adhesion to the walls of the vessels. The next step is formation of cracks in vascular endothelium, leading to an increase in filtration of plasma with lymphocytes, macrophages and plasma cells into surrounding tissue. Increase in the volume of fluid in the tooth chamber contributes to swelling of the pulp and increased pressure in the tissue causing irritation of nerve endings, which in consequence causes pain. Swelling also contributes to venous clamp and impossibility to drain excess of fluid, which leads to further increase in pressure and, consequently, to necrosis of the pulp because of tissue hypoxia. Pressure increase in the chamber is associated with irritation of nerve endings, causing on the one hand increased capillary flow, on the other hand activation of neurokinin increasing pressure in the vessels. During pulp inflammation hemorrhage, destruction of odontoblasts and inflammatory infiltration of lymphoid cells occur.[7,10,11]

Studies have shown that the presence of bacteria in dentinal tubules reduces tubular fluid, contributing to growth of bacteria and increased concentration of toxic substances that lead to tissue destruction.[3]

When the inflammatory process is long-lasting, a tooth-like granulation tissue will appear within the chamber. The tissue is characterized by a large number of blood vessels, cells and collagen fibers. The primary role of granulation tissue is to repair damaged tissue.

Untreated inflammation of the pulp leads to apical inflammation. The pathological process spreads via blood vessels. Initially the vessels unclench, their permeability increases and cells and inflammatory mediators pass into periapical space.[10,11]

2. Aim

The aim of this study was measurement of the width of the root dentinal tubules of necrotic teeth with chronic inflammation of periapical tissues, after endodontic treatment as well as differences related to age and gender.

3. Material and methods

The study material comprised 53 teeth (30 molars, 23 premolars) extracted in patients at the Oral Surgery Department, Medical University of Lublin. The teeth were extracted in patients reporting no general disease.

The teeth were divided into two groups. The examination group (Group W) consisted of 40 teeth extracted because of periapical inflammatory lesions (17 gained from females, 23 from males). Patients included in the group aged between 20

Table 1 – Mean dentinal tubule diameter.

	Group W		Group C		Statistical significance*
	Mean	SD	Mean	SD	
Tubule diameter close to root canal, μm	4.89	0.56	3.10	0.31	0.00**
Tubule diameter close to dentinocemental junction, μm	3.68	0.72	1.38	0.35	0.00**

* According to *U* Mann–Whitney test.
** Statistical significant.

and 74 years. The mean age was 40 years. In the group 15 teeth had been treated endodontically before.

The control group (Group C) consisted of 13 healthy teeth (8 premolars, 5 molars) without caries extracted for orthodontic reasons in 13 patients (8 females, 5 males) aged between 12 and 25 years. The mean age was 16.4 years.

The differences in age between the groups resulted from the inability to obtain clinically healthy teeth without caries and with healthy periodontium from middle-aged and elderly people.

The teeth used in the study were washed in running water, hand-cleansed of any remnants of periodontium, washed in distilled water and dried at room temperature. In order to obtain proper samples the teeth were crushed in a ceramic mortar in liquid nitrogen. Afterwards the pieces were dried in a vacuum chamber and covered with palladium and aurum by means of a sputter coater. Scanning electron microscope pictures were taken in the same direction. The diameter of dentinal tubules of the root was measured at two locations – near the root canal and close to the dentinocemental junction.

The examination was carried out using Scanning Microscope BS 301 Tescan (Tesla, Czech Republic). The research was conducted in the Laboratory of Department of Analytical Chemistry and Instrumental Analysis, Maria Curie-Sklodowska University, Lublin, Poland.

The study was developed using the Statistica 6 (StatSoft, USA) using a non-parametric *U* Mann–Whitney test for comparison of two variables, Kruskal–Wallis test and Spearman's rank correlation. The significance level was $P < 0.05$.

4. Results

SEM pictures showed cross-sections of dentin with dentinal tubules. Different shape of the tubules (round or oval) was probably due to uneven shape of dentinal surface.

In the group of teeth with periapical lesions (Group W) diameter of tubules around the root canal was average 4.89 μm and close to dentinocemental junction 3.68 μm. The maximum diameter measured close to the root canal was 6.3 μm, and close to the dentinocemental junction 4.0 μm. The minimum values for both measurements were 4.5 μm and 1.6 μm. The diameter of the tubules in the teeth of the control group (Group C) was significantly smaller than the tubules in necrotic teeth (Group W). The diameter of dentinal tubules in Group C near the root canal was 3.10 μm and close to the dentinocemental junction 1.38 μm. The maximum diameters were 3.6 μm close to the root canal and 1.9 μm near the dentinocemental junction.

The differences between the diameter of dentinal tubules in the teeth of the control group (Group C) and teeth with periapical lesions (Group W) were highly statistically significant (Table 1). Figs. 1–4 show dentin in the electron microscope, location and size of dentinal tubules and the place of measurement of the diameter close to the root canal and close to the dentinocemental junction in the teeth of both groups – W and C.

As in Group W there were 15 teeth after endodontic treatment, the next step of the research was to observe whether root canal treatment had an effect on the width of dentinal tubules in the roots of the teeth. Comparison of dentinal tubules diameters within the Group W related to endodontically treated teeth and untreated teeth.

In Group W the diameter of dentinal tubules in endodontically treated teeth was higher than in the group untreated teeth, both measured close to the root canal and the cement–dentin border. Average diameter of dentinal tubules in endodontically treated teeth was 3.85 μm for dentin close to the border with cement (maximum 4.7 μm, minimum 3.8 μm) and 5.09 μm close to the root canal (maximum 6.3 mm, minimum 4.3 μm). In endodontically treated teeth in Group W the average diameter of dentinal tubules in the same

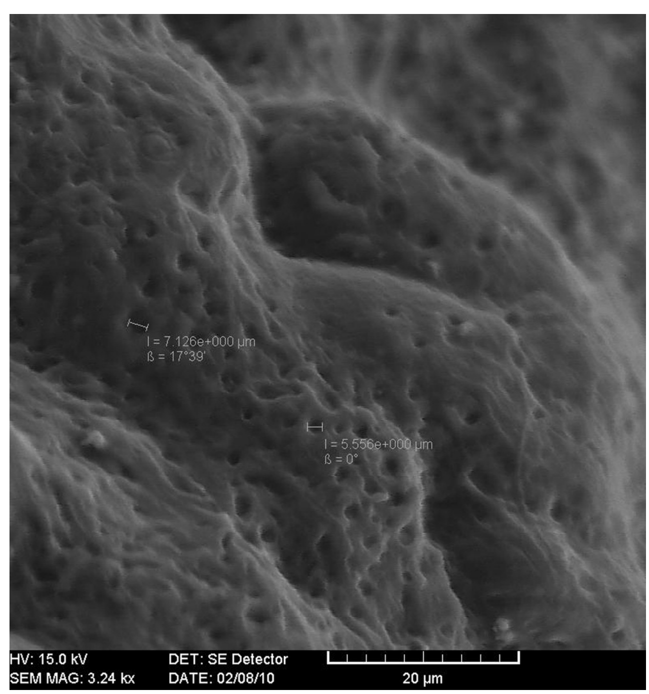

Fig. 1 – SEM picture of tooth 41, Group W (magnification 3 240×).

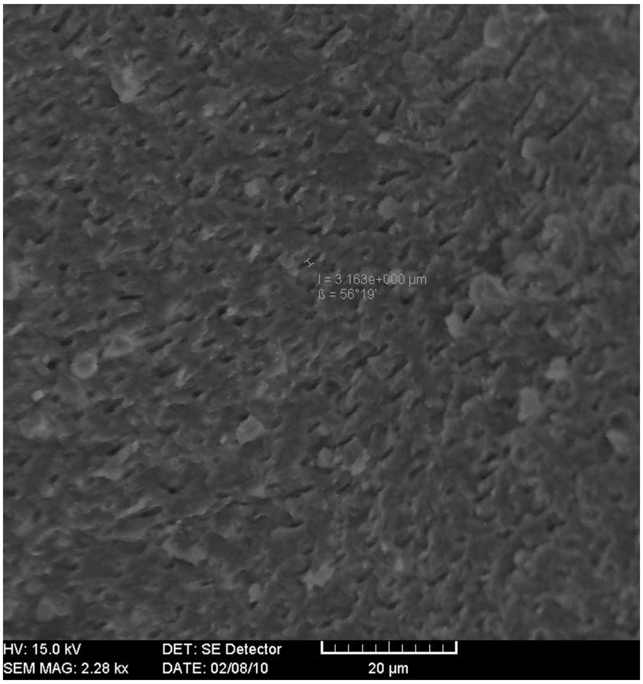

Fig. 2 – SEM picture of tooth 34, Group W (magnification 2 280×).

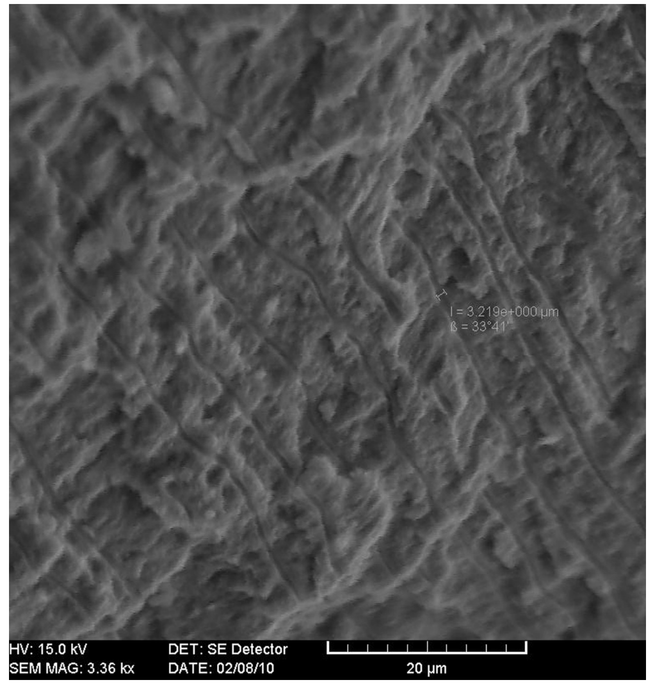

Fig. 3 – SEM picture of tooth 24, Group C (magnification 3 360×).

position was 3.58 µm respectively (maximum 4.9 µm, minimum 1.6 µm) and 4.77 µm (maximum 5.6 µm, minimum 3.5 µm). The differences in the two subgroups teeth were not statistically significant. Table 2 and Fig. 2 show the relationship between the diameter of dentinal tubules in endodontically treated teeth and untreated teeth in Group W (Table 2).

Analysis of the relationship between patient age and the width of the dentinal tubules in necrotic teeth (Group W) showed that the diameter of dentinal tubules was bigger both near the cement–dentin border and close to the root canal with age compared to Group C. However, statistical analysis showed no significant correlation between age of the patients and the width of dentinal tubules in both groups (Table 3).

In further studies the relationship between the diameter of dentinal tubules and gender was examined.

In Group W, 17 teeth were obtained from women aged 20–74 years (mean age 41.7 years) and 23 were obtained from men aged 26–59 years (mean age 38.7 years). In Group C, 8 teeth were gained from women aged 12–18 years (mean age 16.5 years) and 5 teeth from men aged 15–18 years (mean age 16.2 years).

In Group W it was observed that tubules lying close to the cement were slightly wider in men than in women – 3.76 µm and 3.57 µm, respectively. The maximum values obtained in both genders were 6.3 µm in men and 5.6 µm in women, and the minimum of 3.9 µm and 3.5 µm. The diameter of tubules close to the root canal was slightly higher in women (4.96 µm) than in men (3.85 µm). The maximum values were 4.9 µm in women and 4.7 µm in men, the minimum values were 2.3 µm and 1.6 µm in men and women. The relationship between the diameter of dentinal tubules and gender was statistically insignificant.

In the control group (Group C) the average diameter of dentinal tubules located near the border of dentin and cement in men was 1.44 µm, and in women was lower and amounted to 1.35 µm. The maximum values were 2.0 µm in men and 1.9 µm in women, the minimum 1.1 µm and 0.8 µm in men

Table 2 – Mean dentinal tubule diameter in Group W.

	Endodontically treated teeth		Untreated teeth		Statistical significance*
	Mean	SD	Mean	SD	
Tubule diameter close to root canal, µm	5.09	0.52	4.77	0.56	0.17**
Tubule diameter close to dentinocemental junction, µm	3.85	0.58	3.58	0.78	0.51**

* According to *U* Mann–Whitney test.
** Statistically insignificant.

Table 3 – Relationship between patient age and tubule diameter.

	Tubule diameter close to root canal	Tubule diameter close to dentinocemental junction
Group W	0.013, P = 0.938**	0.109, P = 0.502**
Group C	0.122, P = 0.177**	0.316, P = 0.687**

** Statistically insignificant.

and women. In tubules near the root canal the situation was reversed – in women the average diameter was slightly higher (3.11 μm) than in men (3.08 μm). The maximum values were 3.5 μm in men and 3.6 μm in women, minimum – 2.5 μm and 2.9 μm in men and women, however, the relationship was not statistically significant (Table 4).

5. Discussion

Assuming that inflammation is accompanied by low pH and increased activity of osteoclast-like phagocytic cells it is possible that dentinal tubules in the root of the tooth change their width. Inflammation may lead to dissolution of hydroxyapatite crystals of dentinal tubules by the formula:

$$Ca_{10}(PO_4)_6(OH)_2 \rightarrow 10Ca^{2+} + 6PO_4{}^{3-} + 2OH^-$$

which may lead to enlargement of the tubules.[11]

The structure of dentin has been described in the literature mainly in terms of physiology of the tooth and physiological changes associated with age as well as pathological changes occurring in hard tissues such as caries disease or attrition. The researchers describe changes in vital pulp caused by pathogenic microorganisms or excessive forces on the tooth. However, in the available literature we found no studies on alterations of dentinal tubules in cases of coexisting chronic periapical inflammation.

Concerning the diameter of dentinal tubules of vital teeth similar results were obtained by Carda. The width of the tubules also depended on the location of the tubule and age of the patient. Tubules near the chamber were broader and were gradually narrowing to the periphery. In the study the diameter of dentin tubules near the pulp was 3–4 μm and decreased to about 1.7 μm close to enamel and close to cement close to 0.6–0.8 μm.[12]

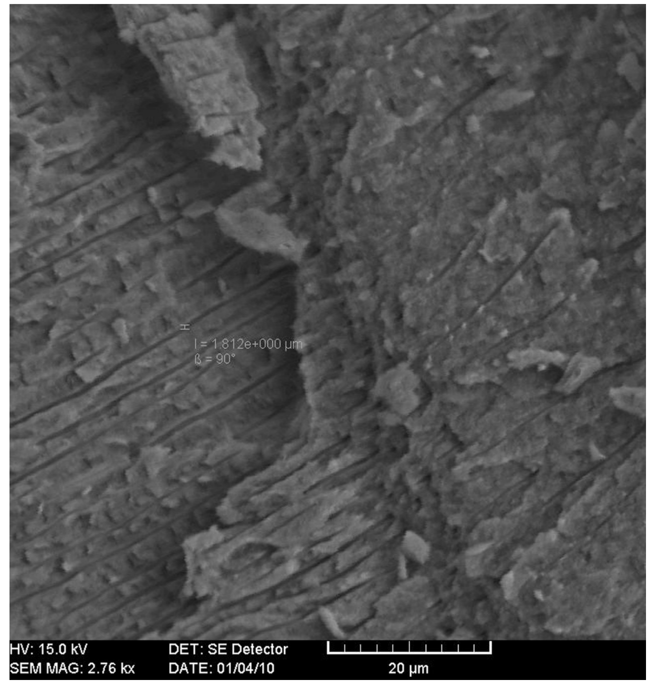

Fig. 4 – SEM picture of tooth 34, Group C (magnification 2 760×).

Lo Giudice et al. claim, that the tubular diameter is larger near to the pulp (3–4 μm) and smaller in the peripheral area near to the DEJ (average diameter 1.7 μm).[13] In our study in vital teeth average diameter of dentinal tubules measured close to the canal was 3.10 μm and close to the cement it was 1.38 μm.

It is believed that changes in width of the tubules of vital teeth are caused by dentin deposition, which takes place in the direction of the periphery of the tissue and therefore close to the enamel and cement tissue becomes 'older' and therefore more mineralized. In addition, in peripheral tubules the layer of peritubular dentin was thicker compared to the area of the chamber, where peritubular dentin did not occur. The diameter of dentinal tubules along the entire length is reduced up to 0.2 μm with age.[10,11,13]

Nalla et al. studied molars in elderly people over 65 years of age. In many places in dentinal tubules they observed loose crystals of hydroxyapatite (HA). HA crystals filled the tubules similar to crystals building peritubular dentin, which is

Table 4 – Relationship between tubule diameter and gender.

	Females		Males		Statistical significance*
	Mean	SD	Mean	SD	
Group W					
Tubule diameter close to root canal, μm	4.96	0.63	4.85	0.51	0.69**
Tubule diameter close to dentinocemental junction, μm	3.57	0.78	3.76	0.68	0.47**
Group C					
Tubule diameter close to root canal, μm	3.11	0.36	3.08	0.25	0.88**
Tubule diameter close to dentinocemental junction, μm	1.35	0.37	1.44	0.34	0.94**

* According to *U* Mann–Whitney test.
** Statistically insignificant.

characterized by high degree of mineralization. In the studied teeth presence of sclerotic dentin depositing with age instead of tubular dentin was also observed. Mean diameter of the tubules ranged from 0.2 μm to 1 μm, and the diameter of the tubules was larger near the pulp tapering gradually toward also observed dentino-enamel junction and dentinocemental junction.[14] Lopes studied the width of dentinal tubules in molars without caries (extracted for orthodontic reasons) in three places: near the pulp, near the border with enamel and dentin of the central body. The widest tubules were adjacent to the pulp (up to 2.99 μm), in the middle of dentin 2.94 μm and near the surface of 2.42 μm.[15]

Ghazali described greater diameter of dentinal tubules in studies on vital molars without caries. He measured the diameter of dentinal tubules near the pulp chamber. The mean diameter tubules obtained in the study was 4.88 μm. Dentin before the measurements was kept in 10% EDTA at pH 7.3 for 4 weeks.[16]

Smaller diameter of dentinal tubules in molars without caries was observed by Ciocca, who prepared samples in a solution of 10% EDTA and 37% phosphoric acid for 15 s. The diameter of the tubules near the pulp was 2.5 μm, and near the dentino-enamel junction – 0.9 μm.[17] The diameters of dentinal tubules described by the two authors were similar to those observed in vital teeth without caries in Group C of our study.

Agematsu examined dentinal tubules in deciduous incisors with attrition but with no visible caries. He observed additional dentinal tubules, running parallel to the tubules typical for healthy tissue. The diameter of the tubules was about 20 μm and was almost 10 times bigger in healthy deciduous teeth without attrition. Tubules of increased diameter were located mostly in the crown and near the dentino-enamel junction. Around the enlarged dentinal tubules a peritubular-like structure was observed as well as collagen fibers arranged along the walls and small spherical structures. The spherical formations were made of calcium phosphate crystals and contained fragments of collagen fibers. In the central part of dentin tubules their walls were concaving, causing distortions of circular cross-section of the tubules. In many locations within the wide tubules bacteria deposited on the walls were observed. In place of bacterial colonization atrophy of peritubular dentin was observed, which may be due to destruction of the walls of tubules by bacteria.[18]

It is believed that under the influence of pathogens and toxic substances at the site of irritation deposition of sclerotic dentin occurs. Sclerotic dentin consists of tubules of smaller diameter and has a higher degree of mineralization. In young people in teeth with inflammation of the pulp under the influence of growth factors deposition of reparative dentin containing dentinal tubules occurs. However, in elderly people newly deposited dentin does not contain tubules, because it is constructed by poorly differentiated cells in place of destroyed odontoblasts.[6,9,19]

6. Conclusions

1. The width of dentinal tubules of root dentin of necrotic teeth with chronic periapical inflammation was significantly higher compared to the teeth with vital pulp.
2. Endodontic treatment does not change the width of root dentinal tubules of root dentin.
3. The width of dentinal tubules of root dentin does not change with age.
4. The width of dentinal tubules of root dentin does not dependent on gender.

Conflict of interest

None declared.

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