



Research Article

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## Morphology and mineral composition of dentine of teeth with a wedge-shaped defect

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### Abstract

Wedge-shaped defects (WSD) are a common pathology with multi-factorial etiology. The study aimed to evaluate the features of the morphological structure and mineral composition of teeth dentine with a WSD to determine their possible relationship with potential etiological factors. Ten maxillary and mandibular human teeth with a WSD extracted for orthodontic purposes and their longitudinal sections were studied using a JSM-6490 LV focused beam electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis INCA Penta FETx3. We studied the mineral composition of dentine in the incisal region (tubercle), surface junction forming a WSD and in 150  $\mu\text{m}$  from it. We identified a percentage of the weight amounts of some elements in the dentine (carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulfur, chlorine, zinc, potassium, and aluminum). The dentine of the teeth with a WSD was characterized by heterogeneity of the morphological structure, which depended on the topography, and the characteristics of the pathological process; it was combined with the changes in the mineral composition. The most pronounced differences in the ultrastructure of dentine were revealed in the area of the surface junction forming the defect, and they were found at a distance of 150  $\mu\text{m}$  from it. We determined a significant difference in the amount of chemical elements in dentine at the surface junction forming the defect and at a distance of 150  $\mu\text{m}$  from it; in the area of the incisal region (tubercle) (with wear facets and without them),  $p \leq 0.05$ . It is impossible to identify one etiological factor responsible for the occurrence of a WSD of the teeth. We think their treatment and prevention will be more effective when we understand the morphological features of hard dental tissues' structure and mineral composition.

**Keywords:** chemical elements, dentine, morphology, non-carious cervical lesions, scanning electron microscope

### 1. Introduction

The high prevalence of non-carious cervical lesions (NCCL) of teeth (60.2% (1), 88.1% (2)) explains a comprehensive study of the issues of their etiopathogenesis. Some authors described their morphology and cross-section shapes (3-6). Recent studies show that enamel is a highly substituted crystalline apatite, but dentine apatite may play a more critical role in regulating ion exchange as well as mineral crystallinity (7). The characteristics of the morphological structure and chemical composition of hard dental tissues describe complicated processes associated with the conditions caused by age and pathology in the body and identify their development (8). Dentine presents physiological exchanges of trace elements after mineralization, and some factors can influence its concentration (9), including cervical pathology. According to Stănuși et al. (10), microcracks precede the onset of non-carious cervical pathology. Microcrack presence disrupts chemical bonds between hydroxyapatite crystals and follow-up penetration of water and other molecules, making a tooth more vulnerable to dissolution, chemical erosion, or abrasive factors (11). But the ideas about the structure of enamel and dentine change over time in some respects, which encourages further research.

Wedge-shaped defects (WSD) are a common form of NCCL (1, 5, 6). Their occurrence increases with the age of patients (1, 2), affecting the microstructure, hardness, and chemical characteristics of dentine (12). According to Levrini

et al. (4), a WSD results from abfraction and has a characteristic macroscopic morphology with many ultrastructural features (3). Igarashi et al. (6) consider their occurrence most likely caused by wear of friction and microstructural loss by stress. The size and depth of the defect are proportional to the intensity and frequency of the applied force (4). In our opinion, the study of a WSD with the help of an electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis makes it possible to assess the peculiarities of the morphology and study the mineral composition (3-5) that is primary in etiopathogenesis. The analysis of the mineral composition of the dentine of the teeth with a WSD was presented in previously published papers (13, 14). Unlike intact samples, there were less sodium, chlorine, and calcium (in the incisal edge [tubercle] and cervical region), sodium, and magnesium (at the equator) detected,  $p \leq 0.05$ . The dentine of the teeth with a WSD contained more oxygen and sulfur (in the incisal edge (tubercle)), phosphorus and zinc (at the equator), carbon and potassium (in the cervical region),  $p \leq 0.05$ . Understanding how chemicals affect dentine apatite structure is of great clinical importance (7). According to Femiano et al. (11), local factors can play only a secondary role. So, it is urgent to identify the characteristics of the morphological structure and distribution of dentine chemical elements of the teeth with a WSD.

The study aimed to evaluate the features of the

morphological structure and mineral composition of teeth dentine with a WSD to determine their possible relationship with potential etiological factors (abrasion, attrition, erosion, abfraction).

## 2. Materials and Methods

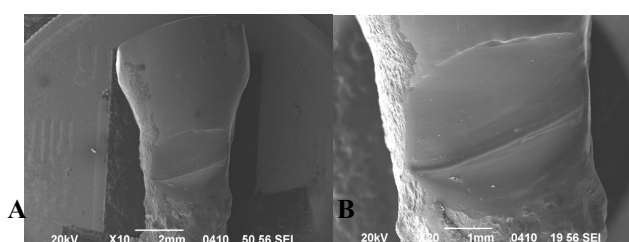
Using a JSM-6490 LV focused beam electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis INCA Penta FETx3 (OXFORD Instruments, England) there were examined ten maxillary and mandibular extracted teeth with a WSD and their longitudinal sections based on previously described method (13). A detailed medical history was collected from the patients whose teeth were examined. A practicing dentist examined these patients. We completed teeth check-ups of patients with no systemic diseases, didn't take regular medications, or had no specific dietary habits. No orthopedic constructions, amalgam fillings, or extracted teeth were in the oral cavity. The teeth were extracted for some orthodontic indications. We studied the morphological structure of the teeth with the help of  $\times 10 \dots \times 5000$  magnification (14). To calculate local mass fractions of chemical elements, we used the peak-to-background ratio method, taking into account matrix corrections for atomic number, fluorescence, and absorption, measured in normal mass percentage (normal mass%). The chemical composition of dentine (in a total of 103 areas) in the incisal region (tubercle), surface junction forming a WSD, and in  $150 \mu\text{m}$  from it was studied. We identified it as a percentage of the weight amounts of carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulfur, chlorine, zinc, potassium, and aluminum. Dentine was examined at approximately the same distance from the enamel-dentine border. The research was performed at the base of the Donetsk Institute of Physics and Technology of the National Academy of Sciences of Ukraine.

Statistical analysis was carried out with the help of the Statistica 12.0 computer program (3BA94C4ED07A). We used the G\*Power program to calculate the sample size. Student's T-test was used to assess the reliability of obtained results. The differences were believed to be statistically significant at  $p \leq 0.05$ . There were averaged replication measurements in one sample before carrying out statistical analysis.

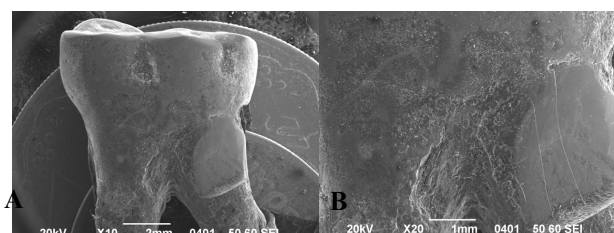
## 3. Results

At the macroscopic level (at magnification up to  $\times 20$ ), the lesion focus had a wedge shape, precise contours, and uneven edges. It was located in the cervical region of the vestibular surface of only coronal part of the tooth (Fig. 1) or spread to the root part (Fig. 2). Noteworthy are the numerous cracks that penetrated the enamel and dentine in the lesion focus (Fig. 2B). Cement lesions with exposed dentine were seen in the area of the lower (near-gingival) surface (Fig. 3). At the mesoscopic level (at magnification of  $\times 100-5000$ ) the dentine surface looked uneven, crater areas were identified in some samples (Fig. 4). Most of the samples had occlusal/incisal wear facets

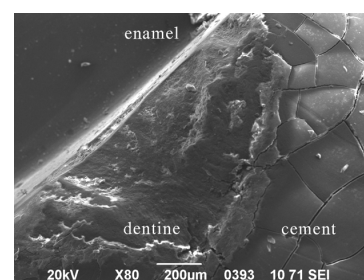
which had an uneven surface and numerous cracks (Fig. 5). Disruptions of the structure of the intertubular substance in the form of cracks and usuras were revealed (Fig. 6). The dentinal tubules were irregular or elongated, with irregular contours (Fig. 7). Organic material was detected on the dentine surface in a deep WSD (Fig. 8), partial or complete obliteration of the lumen of the dentinal tubules was observed depending on the area of the study (Fig. 9). Partial obliteration was more often identified on the upper (coronal) and lower (near-gingival) surfaces forming the defect (Fig. 9A). Complete obliteration was characteristic of the dentine area that was located at the surface junction forming a WSD (Fig. 9B). In the same area foci of demineralization were more often detected where it was rather difficult to identify the structural elements of dentine (Fig. 10). Dentine acquired a dense structure only at a distance of more than  $150 \mu\text{m}$  from the defect.



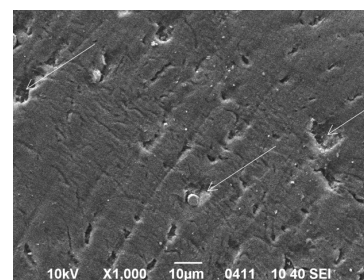
**Fig. 1.** WSD in the cervical region of the tooth A. Magnification  $\times 10$  B. Magnification  $\times 20$



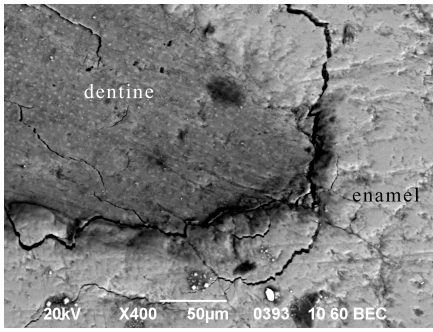
**Fig. 2.** WSD in the region of the distal root of the mandibular molar A. Magnification  $\times 10$  B. Magnification  $\times 20$



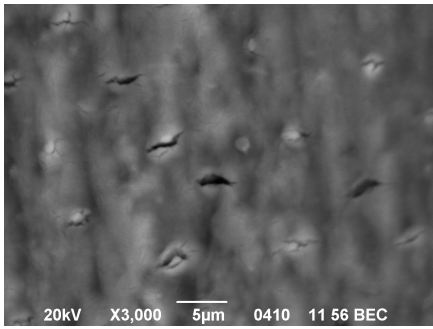
**Fig. 3.** Cement damage with exposed dentine in the area of the lower (near-gingival) surface of a WSD (magnification  $\times 80$ )



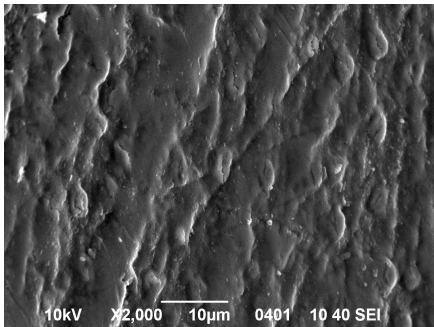
**Fig. 4.** 'Cratered' features on the dentine surface (magnification  $\times 1000$ ) (arrowed)



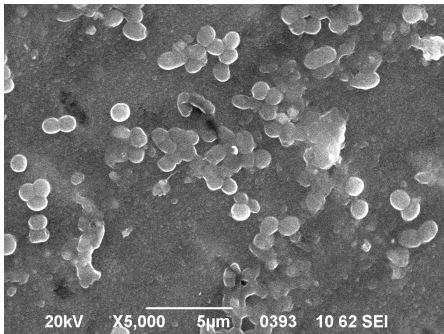
**Fig. 5.** Surface of wear facet (magnification x400)



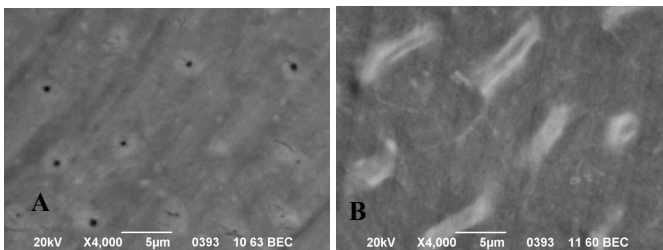
**Fig. 6.** Changes in the structure of the intertubular substance (magnification x3000)



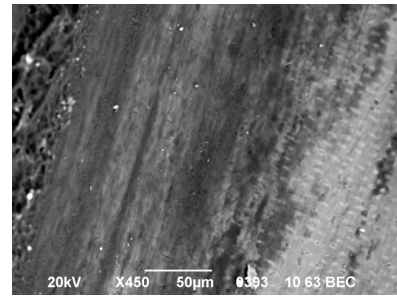
**Fig. 7.** Dentinal tubules in the area of a WSD (magnification x2000)



**Fig. 8.** Organic material on the dentine surface (magnification x5000)

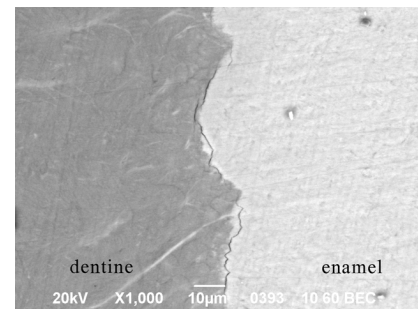


**Fig. 9.** Obliteration of the lumen of the dentinal tubules in a WSD (magnification x4000) A. Partial obliteration of dentinal tubules B. Complete obliteration of dentinal tubules

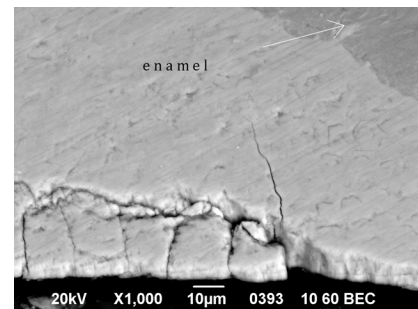


**Fig. 10.** Dentine at the surface junction forming a WSD (magnification x450)

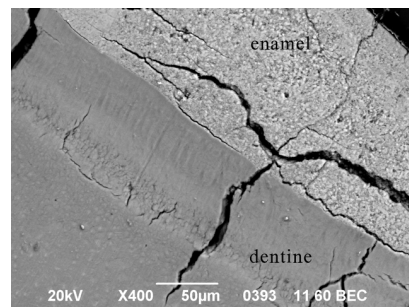
A disruption of the dentine-enamel bonding with the formation of micro-crevices at the dentine-enamel junction was determined on thin longitudinal sections (Fig. 11). The enamel penetrated the dentine in some specimens (Fig. 12). The sections revealed internal microcracks that passed from enamel to dentine (Fig. 13). In dentine with occlusal/incisal wear facets there were identified the areas of sclerotic dentine where the areas of partial and complete obliteration of the lumen of the dentinal tubules alternated which had the wrong direction or were completely absent (Fig. 14).



**Fig. 11.** Microcrevices at the dentine-enamel junction, longitudinal section (magnification x1000)

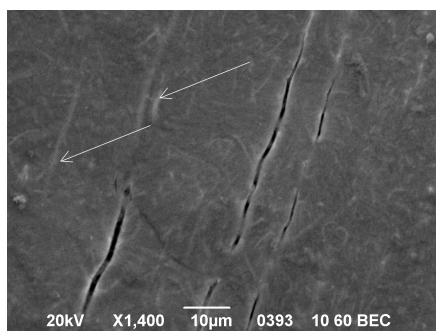


**Fig. 12.** Penetration of enamel into dentine, longitudinal section (magnification x1000)



**Fig. 13.** Microcracks in enamel and dentine, longitudinal section (magnification x400)





**Fig. 14.** Sclerotic dentine area, longitudinal section (magnification x1400) (arrowed)

The mineral composition of dentine was determined in the area of the surface junction forming a WSD, where the greatest morphological changes were detected and its comparison with dentine at a distance of 150 µm from the defect (Table 1). Significant differences were obtained in carbon, oxygen, magnesium, phosphorus, and zinc content depending on the study area,  $p \leq 0.05$ . In the area of the surface junction forming a WSD, there was less oxygen, magnesium, phosphorus, zinc, and more carbon,  $p \leq 0.05$ .

**Table 1.** Mineral composition of dentine in the area of the surface junction forming a WSD and at a distance of 150 µm from it

Chemical element (normal mass %)	Dentine at the surface junction forming a WSD ( $X \pm m$ )	Dentine in 150 µm from the surface junction forming a WSD ( $X \pm m$ )	P
C	32.47±9.3678	25.09±2.1645	<0.0001*
O	33.76±4.0247	36.16±2.0153	0.0041*
Na	0.34±0.1465	0.39±0.1152	0.1948
Mg	0.19±0.1628	0.42±0.1751	<0.0001*
Al	0.03±0.0427	0.04±0.0547	0.7138
P	11.34±2.6162	13.55±0.5866	<0.0001*
S	0.17±0.1903	0.17±0.1726	0.8683
Cl	0.11±0.1234	0.09±0.0665	0.0885
K	0.05±0.0659	0.02±0.0333	0.1021
Ca	21.44±5.7701	23.89±0.6777	0.4010
Zn	0.15±0.1694	0.29±0.2966	0.0126*

\* Statistically significant

**Table 2.** Mineral composition of dentine in the area of incisal region (tubercle) and teeth with a WSD

Chemical element (normal mass %)	Dentine with occlusal/incisal wear facets	Clinically intact dentine	P
C	38.36±0.8508	27.52±1.5732	<0.0001*
O	29.24±0.5804	36.54±0.6683	<0.0001*
Na	0.39±0.0612	0.36±0.0644	0.3698
Mg	0.14±0.0438	0.44±0.0817	0.0000*
Al	0.02±0.0120	0.02±0.0232	0.6909
P	11.17±0.2656	12.46±0.5450	<0.0001*
S	0.11±0.0275	0.10±0.0501	0.6103
Cl	0.14±0.0424	0.09±0.0414	0.0668
K	0.10±0.0330	0.02±0.0228	<0.0001*
Ca	20.14±0.5114	22.46±1.0749	<0.0001*
Zn	0.21±0.1144	0.05±0.0572	<0.0001*

\* Statistically significant

A comparative analysis of the mineral composition of dentine in the region of the incisal region (tubercle) was also carried out (Table 2). Significant differences were found in the

carbon, oxygen, magnesium, phosphorus, potassium, calcium, and zinc content,  $p \leq 0.05$ . Dentine with occlusal/incisal wear facets differed in a smaller amount of calcium, magnesium, phosphorus, and oxygen and a large amount of carbon, potassium, and zinc,  $p \leq 0.05$ .

#### 4. Discussion

The morphological features of the enamel of the teeth with a WSD were described in previously published papers (15). According to Michael et al. (3) and Hayashi et al. (16), short periods of activity and longer periods of stability can be distinguished in the development of NCCL. The active phase is characterized by smooth defect surfaces and clear contours. During it, open dentinal tubules are identified morphologically, while hyperesthesia symptoms are detected clinically (4). The period of stability corresponds to closed dentinal tubules due to the formation of sclerotic dentine with increased density (4). Therefore, depending on the stage of the pathological process, the number of exposed dentinal tubules can vary (3). Probably, it explains the different degrees of obliteration of the lumens of the dentinal tubules developing as a defense mechanism of the body in the specimens with a WSD (17). Chistyakova et Petrouk (18) observed obliteration of the dentinal tubules along the entire length of exposed dentine. According to Daley et al. (19), the differences in the diameter of open and sclerotic tubules at different dentin levels are associated with intratubular dentine deposition. Yan et al. (20) explain the disturbances in intratubular mineralization by an imbalance of calcium and phosphorus ions.

“Dead tracts” were revealed in the dentine of teeth with NCCL by Walter et al. in 88% of the cases (62% were directly in the lesion focus). Sclerosed dentine was observed in the lesion focus of the teeth in 48% (5). According to Abou Neel et al. (21), secondary and tertiary dentine formation is associated with its ability to regenerate. The research results of Tkachenko et al. (8) indicate the presence of hypermineralized dentine in the affected area with stenosis and obliteration of the lumen of the dentinal tubules. The researchers found the greatest changes in the dentine morphology of the teeth with a WSD at the surface junction, forming the defect. The changes spread at a distance up to 150 µm from it. According to the point of view of Guimarães et al. (22), it is connected with higher stress levels focused at the lesion zenith and in the entire cervical region of the tooth (23).

The areas of demineralization were identified in the teeth where the depth of the defect was within the dentine contributing to the formation of NCCL at an early stage, according to Nascimento et al. (24). This is due to the water content in the dentine, through which the diffusion of acid to the mineral components is carried out (25). During demineralization, calcium is released (21), which we observed in the teeth with a WSD (13). Replacing calcium with other chemical elements increases the vulnerability of hard tissues to the effects of acids and their solubility (21). The subsequent



loss of dentine minerals and occlusal attrition contributes to the further development of the pathology and can be considered an etiological factor (26). The revealed significant differences in the amount of sodium in all studied areas of teeth indicate its important role in maintaining the normal state of dentine (27). The significantly higher content of sulfur in the area of the incisal edge (tubercle) and potassium in the cervical region ( $p \leq 0.05$ ) (14) is probably due to the presence of wear facets (2) and microcracks in the enamel (15) of the teeth with NCCL. According to Fernández-Escudero et al. (9), the increase in the concentration of sulfur and potassium in coronal dentine is age-related and does not depend on sexual identity. The development of wear facets in combination with microcracks in teeth with a WSD confirms that occlusal stress is the main etiological factor and the root cause of the development of this NCCL form (11). Badavannavar et al. (25) consider the presence of pathological wear and microcracks to be a manifestation of abfraction. According to Worawongvasu (28), abrasion and erosion are combined etiological factors in the formation of NCCL. Significant differences in the amount of magnesium and zinc indicate their influence on the morphology, crystallinity, and solubility of dentine apatite (7).

The dentine surface of some samples, like the enamel (15), had crater areas and organic material, which agreed with the data (3, 4). The development of crater areas is associated with the effect of corrosion factors (acid attack), or it is considered a sign of abfraction. The presence of organic material indirectly indicates the roughness of the dentine surface. Numerous cracks in the lesion focus of the teeth with a WSD were described in the publications as the most common in this form of NCCL and as a result of abrasion or abfraction (3, 4). The cracks in the dentine did not have a clear direction associated with the peculiarities of its structural organization (29) or multi-factorial etiology (3). It is possible that they may be an artifact (3).

Longitudinal sections revealed internal microcracks with numerous branches passing from enamel to dentine. Similar results were described by Stănuși et al. (10). But dentine is able to withstand significant loads and perform its functions even in the presence of a large number of cracks and significant damage. The penetration of enamel into dentine revealed in the study in some areas of the dentine-enamel junction was called “enamel bridges,” which are involved in the maturation of enamel and dentine (12).

In conclusion, obtained results made it possible to clarify the microstructure of dentine in the wedge-shaped form of NCCL, and determine the features of its mineral composition. But it is impossible to identify one etiological factor responsible for the occurrence of a WSD of the teeth. They are probably an external manifestation of the combination of abrasion, attrition, erosion, and abfraction. In our opinion, the treatment and prevention of a WSD will be more effective when understanding the morphological features of hard dental

tissues' structure and chemical composition, which justifies further research.

### Conflict of interest

The authors declared no conflict of interest.

### Funding

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### Acknowledgments

None to declare.

### Authors' contributions

Concept: I.I.Z., Design: I.I.Z., Data Collection or Processing: I.I.Z., Analysis or Interpretation: I.I.Z., Literature Search: I.I.Z., Writing: I.I.Z.

### Ethical Statement

The study was carried out based on the principles of the WMA Declaration of Helsinki Ethical Principles for Medical Research Involving Human Objects as amended in 2013, Order No. 690 of the Ministry of Health of Ukraine (dated September 23, 2009) and approved by the Bioethics Commission of Donetsk National Medical University (No 43, dated January 21, 2021). Before the study, written informed consent was taken from all the participants.

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