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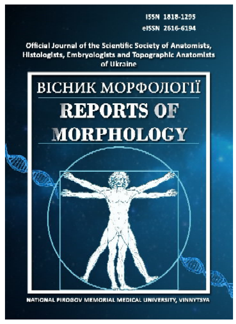

Morphological study of hard dental tissue condition with various types of dental deposits

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Today, it has been established that the primary factor in preventing dental caries is controlling the level and type of dental deposits. However, the literature has not yet addressed the specific morphological changes in the hard dental tissues when non-mineralized and mineralized dental plaque is present on the tooth surface. The aim of this study was to examine the microscopic structure of the cuticle and hard dental tissues and to assess the X-ray spectral changes associated with different types of dental deposits. Epimicroscopic and X-ray spectral examinations were performed on 32 teeth with various types of supragingival deposits, extracted for orthodontic and surgical indications. Each tooth was cut in the vestibulo-oral direction using a diamond bur at low speed, then one half was polished, stained with PAS-Alcian blue, and photographed at different magnifications in epiprojection. Samples were carbon-coated in a vacuum chamber, and the inorganic components in the cuticle and mineralized deposits were assessed using the Link AN 10.85s micro X-ray analyzer. Thin sections of the other tooth halves were prepared, stained with PAS-Alcian blue, and examined under the "Olympus" microscope at various magnifications. Statistical analysis was performed using standard parametric methods with "Statistica 6.0" and "Microsoft Excel 2002" software. Significant findings in the hard dental tissues included the presence of non-mineralized (soft plaque, dental biofilm), mineralized (dental calculus), and smoker's plaque. In areas with soft plaque, enamel prisms remained intact, with no changes in the enamel-dentin junction or dentin, though the cuticle was nearly absent. In areas with dental biofilm, enamel thickening, the absence of characteristic enamel prism patterns, fragmentation of the reticular layer, and formation of interglobular dentin were observed. Morphological changes in mineralized deposits included enamel thinning, prism disorganization, localized hypermineralization, destroyed enamel-dentin junctions, and "dead tracts" in the dentin. In cases with smoker's plaque, nicotine pigment accumulated in Retzius line biomineralization zones, along with thickening of the reticular layer and "dead tracts" in the deeper dentin layers. A comparison of the main inorganic components (Ca, P, Mg, Ca/P) in the cuticle and mineralized deposits showed significant differences in phosphate, magnesium, and Ca/P ratios, indicating disrupted metabolic processes at the "enamel-oral fluid" interface, promoting calculus formation. Thus, the morphological and X-ray spectral changes observed in the enamel and surrounding tissues provide an objective assessment of tissue condition with various types of deposits. These findings should be considered in selecting a scaling method.

Keywords: *mineralized and non-mineralized dental deposits, morphological study.*

Introduction

The study of the etiology [25, 26, 27, 33] and pathogenesis [6, 14, 19, 31] of dental caries has a long history. Dentists' ongoing focus on this issue is associated with the widespread prevalence of dental caries globally [5, 17, 21, 29]. Currently, dental biofilm (a type of dental plaque) is considered a monoetiologic factor in the development and progression of dental caries [12, 22, 24, 34] and periodontal diseases (gingivitis, periodontitis) [1, 3, 11, 18]. According to many researchers [2, 7, 9, 23], colonies of oral microorganisms on the tooth surface within dental plaque are a fundamental cause of the destruction of both hard dental tissues and the soft and bone tissues of the periodontium.

To date, numerous morphological studies have investigated the role of various types of mineralized and non-mineralized dental deposits (soft plaque, dental biofilm, calculus, and smoker's plaque) in the etiology and pathogenesis of periodontal diseases and dental caries [12, 13, 34]. However, there is a lack of morphological studies focused on the condition of tooth enamel, its covering structures, particularly the cuticle, as well as the enamel-dentin junction and dentin, in the presence of different types of dental deposits.

According to reports by authors [32], immobilized alkaline phosphatase on the enamel surface retains its enzymatic activity, which contributes to the regulation of metabolic processes in enamel at the "enamel-saliva" interface. Alkaline phosphatase on the enamel surface binds calcium and phosphate ions, forming high concentrations that impact the processes of enamel mineralization and remineralization. Consequently, it is necessary to study the composition of inorganic chemical elements in the surface structures of enamel, as this is an indicator of the stability and resistance of hard tissues to potential damage during the application of different dental plaque removal methods, especially in individuals highly susceptible to dental caries. Additionally, the role of inorganic compounds and ions on the enamel surface in the formation of mineralized plaque has not been clarified.

The aim of this study is to investigate the microscopic structure of the cuticle and hard tissues of the tooth and to characterize X-ray spectral changes in the presence of various types of dental deposits.

Material and methods

The study was conducted under the initiative of the Department of Orthodontics at Shupyk National Healthcare University of Ukraine, titled "An Integrated Approach to the Diagnosis and Treatment of Orthodontic Patients with Comorbidities," in compliance with the ethical principles of the Declaration of Helsinki. The study was approved by the Ethics and Academic Integrity Committee of Shupyk National Healthcare University of Ukraine (Protocol No. 6/27 dated 28.06.2024).

To achieve the study's objectives, 32 teeth from the mandible (4 incisors, 6 canines, and 22 premolars and molars) with various supragingival dental deposits were examined. These teeth were extracted based on orthodontic and surgical indications. After extraction, the teeth were stored in a 10 % neutral formalin solution. The sample groups were distributed as follows: the first group (2 teeth) had soft plaque, the second group (10 teeth) had dental biofilm, the third group (10 teeth) had dental calculus, and the fourth group (10 teeth) had smoker's plaque. Five intact teeth without deposits, extracted for orthodontic reasons, served as controls.

Each tooth was sectioned in a vestibulo-oral direction into two halves using a diamond bur at low speed. One half of each tooth was polished and histochemically stained with PAS-Alcian blue. The crown fragments were then photographed at different microscope magnifications in epi-projection. Next, they were coated with carbon in a vacuum chamber, and the content of inorganic components in the cuticle and mineralized dental deposits was analyzed. The chemical composition of these structures was examined at the Kharkiv Research Institute "Monocrystal" using a Link AN 10.85s micro-X-ray analyzer.

Thin sections (15-20 microns) were prepared from the second half of each sample tooth and stained with PAS-Alcian blue. Different areas of these sections were studied and photographed at various magnifications using an "Olympus" microscope.

Statistical analysis of the inorganic component content in the cuticle and mineralized dental deposits was performed using standard parametric methods with "Statistica 6.0" and "Microsoft Excel 2002" statistical software. Mean values and standard errors were calculated. The significance of differences between the results was determined using Student's t-test [20].

Results

The first stage of the study was devoted to examining the histological structure of the enamel of intact teeth without dental deposits. Some specific features of the enamel structure were observed in the equator and cervical regions of the tooth. In particular, the enamel in the cervical region of the tooth crown is very thin but covered by a thick layer of cuticle (Fig. 1).

Figure 2 shows the structure of the enamel of an intact tooth without dental plaque. We have established that the outer layer of the cuticle contains alcian-positive structures,

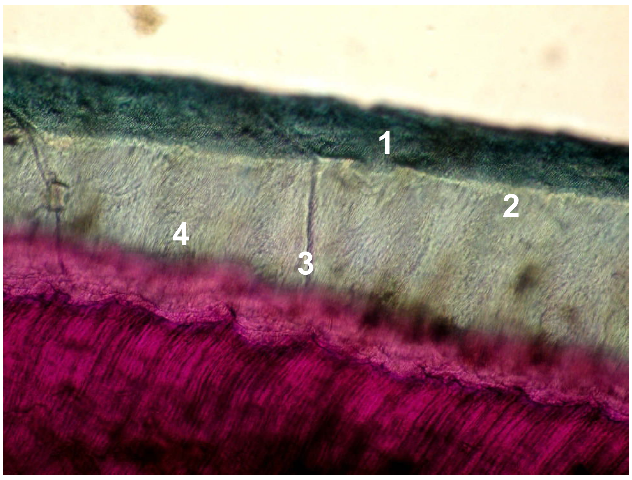

Fig. 1. Structure of the cuticle: 1 - outer layer of the cuticle; 2 - inner layer of the cuticle; 3 - lamella; 4 - enamel prisms. PAS-Alcian blue. x400.

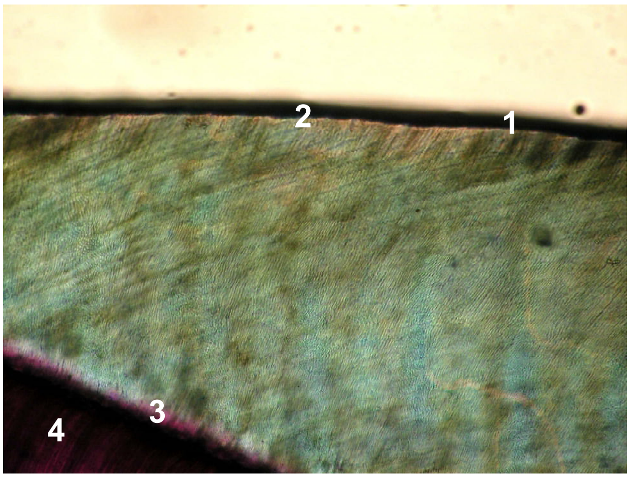

Fig. 2. Structure of enamel: 1 - cuticle; 2 - enamel; 3 - reticular layer; 4 - dentin. PAS-Alcian blue. x400.

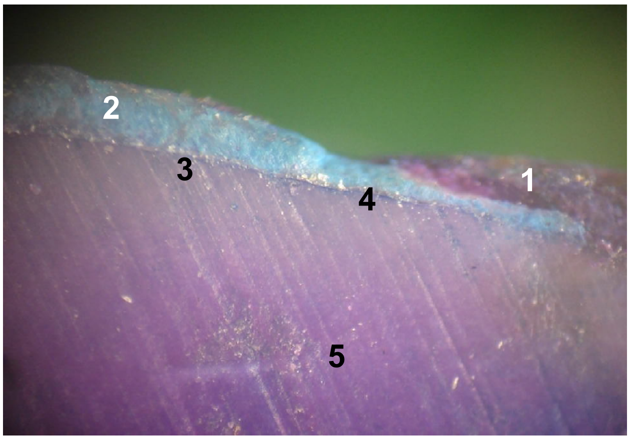

Fig. 3. Soft dental plaque in the cervical area: 1 - dental plaque; 2 - enamel; 3 - enamel-dentin junction; 4 - reticular layer; 5 - dentin. PAS-Alcian blue. x16.

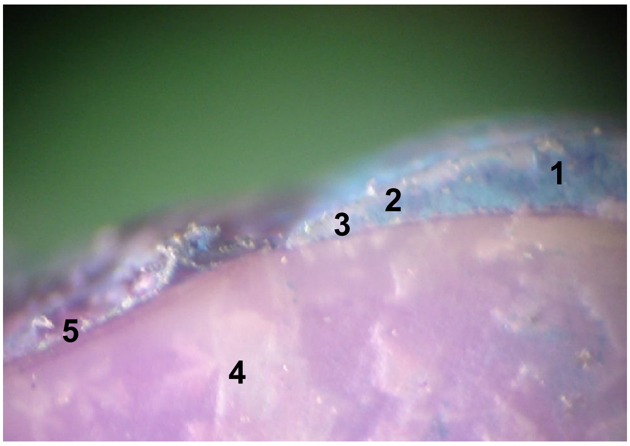

Fig. 4. Dental plaque: 1 - preserved enamel; 2 - destroyed enamel areas; 3 - fragmentation of the reticular layer; 4 - interglobular dentin; 5 - areas of destroyed cementum. PAS-Alcian blue. x16.

which include acidic glycosaminoglycans. Meanwhile, the inner layer consists of PAS-positive glycoproteins. In the cervical region of the tooth, the cuticle appeared as a thin strip, stained black. The inner layer (Nasmyth's membrane) has no distinct boundaries and merges into the enamel prisms. The entire thickness of the enamel (from the cuticle to the enamel-dentin junction) is permeated by well-defined lamellae, to which the Tomes' fibers, located in the dentinal tubules, are attached and stained red. In the equatorial region, the enamel is thickened, consisting of bundles of enamel prisms arranged in an S-shape (Hunter-Schreger bands) and stained blue. At a 30 ° angle to the cuticle, Retzius lines, stained brown, are visible. A faintly expressed network layer is located along the enamel-dentin junction.

In the second stage of the research, the morphological structure of different types of dental deposits and their inorganic chemical content were studied. Thick sections stained with PAS-alcian blue revealed the following types of dental deposits: soft dental plaque, dental plaque, dental calculus, and smoker's plaque.

Soft dental plaque appears in the cervical region of the tooth as a narrow strip stained in lilac (Fig. 3). The enamel structure and the pattern of enamel prisms remain intact, and the cuticle is almost completely absent on its surface. The enamel area is stained blue, dentin is stained pink, and cement is stained lilac. The structure of the enamel-dentin junction and dentin is undisturbed. The dentinal tubules, appearing as red parallel structures, have a clear direction.

In the presence of dental plaque in the cervical region of the tooth (Fig. 4), the enamel is thickened, and in its central part, the pattern of enamel prisms is absent. The prisms are replaced by pale pink homogeneous structures. Bundles of enamel prisms are preserved in the area of the enamel-dentin junction, but its boundary is disrupted in many areas (fragmentation of the reticular layer). Microscopic examination revealed changes in the dentin: the formation of interglobular dentin, which is stained white-pink.

In the area of formed supragingival dental calculus, enamel thinning is observed, which, when stained with PAS-Alcian blue, appears as a thin strip of blue color. In the

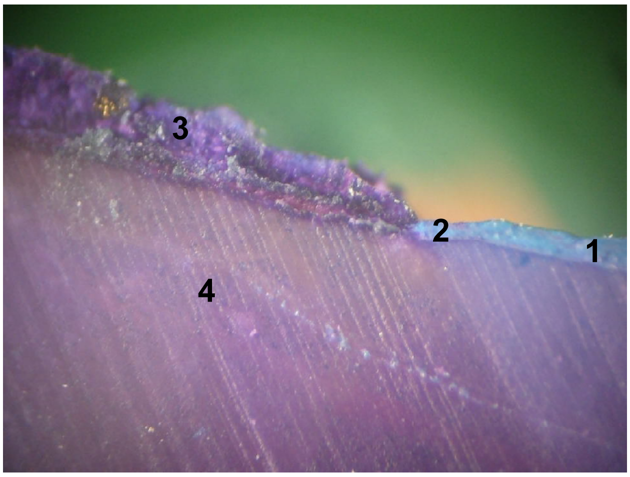

Fig. 5. Supragingival dental calculus: 1 - preserved enamel; 2 - destroyed enamel-dentin junction; 3 - dental calculus; 4 - "dead tracts in dentin". PAS-Alcian blue. x16.

Fig. 6. Smoker's dental deposits: 1 - preserved enamel; 2 - dental calculus with nicotine pigment; 3 - destroyed enamel-dentin junction; 4 - dentin. PAS-Alcian blue. x16.

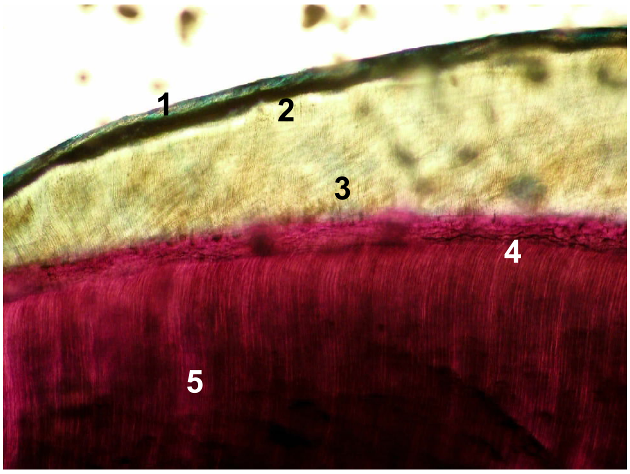

Fig. 7. Smoker's plaque: 1 - outer layer of the cuticle; 2 - inner layer of the cuticle; 3 - Retzius lines; 4 - reticular layer; 5 - dead tracts in dentin. PAS-Alcian blue. x400.

area of dental calculus, the enamel-dentin junction is completely destroyed. Dentin exhibits destruction of dentinal tubules and the formation of "dead paths", which, according to Hasyuk A. P. and co-authors [13], result from the fragmentation of odontoblast processes or their complete destruction (Fig. 5).

The most pronounced destructive changes in the enamel and dentin of the tooth were observed during microscopic examination of teeth with smoker's plaque, likely related to the toxic effects of nicotine pigment. Changes in the coloration of all components of the tooth's hard tissues were noted, presumably due to the penetration of nicotine pigment into the enamel and dentin (Fig. 6). On histochemically stained tooth sections, the preserved enamel is light green, dentin is dark orange, and the smoker's plaque is light yellow or dark brown.

A more detailed study of the nicotine pigment penetration level into the tooth tissues was conducted on thin sections under a microscope at x400 magnification (Fig. 7). When stained with PAS-Alcian blue, the outer layer of the cuticle appears as a light blue strip, while the Nasmyth's membrane (inner layer) appears dark brown. Pronounced changes were observed in the tooth enamel: most of the terminal sections of the enamel prisms are light yellow or brown, with intense nicotine pigment deposits in the Retzius lines. Additionally, the mesh layer at the dentin boundary is significantly thickened. Numerous "dead paths" were detected in the deep layers of the dentin.

The content of the main mineral components in the tooth cuticle and mineralized dental plaque, determined through micro X-ray spectral analysis, is presented in the table (Table 1). A comparison of the obtained results revealed significant differences in the ionic composition of the studied inorganic components. Specifically, the calcium ion (Ca^{2+}) content in the mineralized dental plaque tended to decrease compared to the tooth cuticle, and the difference was not statistically significant ($p>0.05$). In contrast, the magnesium cation level in the dental plaque was elevated and differed significantly from that in the cuticle with a high degree of reliability ($p<0.001$). It is noteworthy that the average phosphate content in the solid dental deposits was significantly higher compared to that in the tooth cuticle ($p<0.001$), while the Ca/P ratio was similarly reduced.

Discussion

The epimicroscopic studies of stained sections from extracted teeth aligned with literature data [11, 15, 16, 30] concerning the primary morphological formations on the tooth surface: soft plaque, dental biofilm, calculus, and smoker's plaque.

Our morphological findings on the structure of hard dental tissues, both in normal conditions and in the presence of non-mineralized and mineralized deposits, confirmed the findings of scientific publications [12, 13]. However, the studies of domestic and international authors [4, 8, 10, 12] have mainly focused on the morphological,

Table 1. Indicators of the content of the main macroelements in the areas of the cuticle of the tooth neck and mineralized dental deposits (tartar).

Field of study	The content of the main macroelements, % of dry weight				
	Ca^{2+}	PO_4^{3-}	Cl	Mg^{++}	Ca/P
Cuticle of the tooth	10.60±0.03	6.042±0.021	0.343±0.113	0.089±0.049	1.754±0.009
Tartar	10.54±0.22	7.381±0.124	0.154±0.024	0.668±0.092	1.352±0.021
p	p>0.05	p<0.001	p>0.05	p<0.001	p<0.001

Note: p - the significance level of the difference in macroelement content between the tooth cuticle and mineralized dental deposits.

biochemical, and microbiological features of dental deposits in the development of dental caries, fluorosis, and periodontal disease [13, 28, 30]. In contrast, our research is the first to establish that all types of supragingival dental deposits induce morphological changes in the tooth's hard tissues and its surface structures: the cuticle, enamel, enamel-dentin junction, and dentin. For instance, in teeth with soft plaque, we observed almost complete absence of the cuticle and no destructive changes in dentin. In extracted teeth with biofilm, the enamel was thickened, showing destruction of its surface prisms and fragmentation of the inner reticular layer. The dentin's response (in the form of excessive interglobular formation) seems to be an adaptive process. In cases with supragingival mineralized deposits (calculus), enamel thinning, prism disorganization, hypermineralization (an adaptive response), and complete destruction of the enamel-dentin junction were observed. The changes in dentin included fragmented and destroyed Tomes' fibers forming "dead tracts", as noted in studies [13] on dental caries progression. More pronounced morphological changes were seen in teeth with smoker's plaque: the presence of nicotine pigment in Retzius lines, slight destruction of enamel prisms, thickening of the aprismatic enamel layer, and "dead tracts" in the deeper dentin layers.

Our findings on the main chemical elements in the cuticle of intact teeth, obtained through micro-X-ray spectral analysis, established a Ca/P ratio of 1.754±0.009, which corresponds to the chemical formula of octacalcium phosphate crystal ($Ca_8(H_3PO_4)_2x5H_2O$) with a negative charge, as reported by [32]. This supports the literature [32], suggesting that this structural formation in teeth represents the initial stage of creating the inorganic matrix of enamel, as in an alkaline environment, octacalcium phosphate in Nasmyth's membrane can convert to hydroxyapatite under the influence of alkaline phosphatase. However, a similar ratio in mineralized plaque (1.352±0.021) corresponds to brushite crystal ($Ca^{2+}HPO_{4-}x2H_2O$) with a positive charge, as noted in [32]. Based on these results, we suggest that in the acidic oral environment, an electrochemical reaction occurs, depositing brushite crystals on the cuticle surface and forming dental calculus.

We believe that the observed decrease in calcium ions within the structure of mineralized dental deposits compared to the tooth cuticle, along with a substantial increase in magnesium ions, may result from their substitution.

Future research should focus on studying morphological changes in enamel and its adjacent tissues following different methods of dental deposit removal to determine the most optimal approach for various types of dental deposits.

Conclusions

1. It has been established that the primary morphological formations on the tooth surface are: soft plaque, dental biofilm, calculus, and smoker's plaque.

2. Morphological analysis revealed the following changes in the hard dental tissues associated with various types of deposits: absence of the cuticle in areas with soft plaque accumulation; enamel thickening, partial fragmentation of the reticular layer, and formation of interglobular dentin in adjacent regions with dental biofilm; enamel thinning, disorganization of enamel prisms, hypermineralization, destruction of the enamel-dentin junction, and "dead tracts" in the dentin with mineralized deposits; accumulation of nicotine pigment in Retzius biomineralization lines, thickening of the reticular layer, and "dead tracts" in dentin with smoker's plaque.

3. The results of morphological and X-ray spectral analyses of extracted teeth with various types of non-mineralized and mineralized plaque indicate the necessity for a differentiated approach to the removal of dental deposits.

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МОРФОЛОГІЧНЕ ДОСЛІДЖЕННЯ СТАНУ ТВЕРДИХ ТКАНИН ЗУБІВ ПРИ РІЗНИХ ВИДАХ ЗУБНИХ НАШАРУВАНЬ

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Сьогодні встановлено, що основним фактором профілактики каріозного ураження зубів являється контроль рівня і характеру зубних нашарувань. Проте, дотепер в літературі не висвітлені питання характеру морфологічних змін у твердих тканинах зуба при наявності на його поверхні немінералізованого та мінералізованого зубного нальоту. Метою проведеного дослідження було вивчення мікроскопічної будови кутикули і твердих тканин зуба та характер рентген-спектральних змін при наявності різних видів зубних нашарувань. Проведено епімікроскопічне та рентген-спектральне дослідження 32 зубів з різними видами над'ясенних зубних відкладень, видалених за ортодонтичними і хірургічними показаннями. Кожний зуб за допомогою алмазної фрези на малих обертах розрізали у вестибуло-оральному напрямку на дві половини, одну з яких полірували, забарвлювали ШИК-альціановим синім і фотографували при різних збільшеннях мікроскопа в епіпроекції. У подальшому напилювали вуглецем у вакуумній камері та визначали вміст неорганічних компонентів в кутикулі та мінералізованих зубних відкладеннях (на мікрорентгенівському аналізаторі Link AN 10.85s). З другої половини зуба виготовляли тонкі шліфи та забарвлювали ШИК-альціановим синім і вивчали при різних збільшеннях мікроскопу "Олімпус". Статистичну обробку результатів проводили з використанням загальноприйнятих параметричних методів із застосуванням пакета статистичних програм "Statistica 6.0" і "Microsoft Excel 2002". У твердих тканинах зубів з різними видами нальоту встановлені такі зміни: наявність немінералізованого (м`який зубний наліт, зубна бляшка), мінералізованого (зубний камінь) та нальоту курця. В ділянках накопичення м`якого зубного нальоту малюнок емалевих призм збережений, змін в емалево-дентинному з'єднанні та дентині не виявлено, проте кутикула майже повністю була відсутня. На ділянках емалі із зубною бляшкою виявлено її потовщення, відсутність характерного рисунку емалевих призм, фрагментація сітчастого шару та утворення інтерглобулярного дентину. Характерними морфологічними змінами при наявності мінералізованих зубних відкладень є: стоншення емалі, дезорганізація емалевих призм і підвищена мінералізація її окремих ділянок, зруйноване емалево-дентине з'єднання та "мертві шляхи" в дентині. У випадку наявності нальоту курця встановлено накопичення нікотинового пігменту в ділянках ліній біомінералізації Ретціуса, а також потовщення сітчастого шару емалі та "мертві шляхи" в глибоких шарах дентину. При порівнянні вмісту основних неорганічних компонентів (Ca, P, Mg, Ca/P) в кутикулі зуба та мінералізованих зубних відкладеннях встановлені істотні розбіжності вмісту фосфатів, магнію та співвідношенні Ca/P, котрі свідчили про порушення обмінних процесів на межі "емаль-ротова рідина", що сприяє утворенню зубного каменю. Таким чином, отримані результати морфологічних та рентген-спектральних змін в емалі зуба й прилеглих тканинах дають можливість об'єктивно оцінити стан досліджуваних тканин при наявності різних видів зубних нашарувань. Результати досліджень необхідно враховувати при виборі методу скейлінга.

Ключові слова: *мінералізовані та немінералізовані зубні нашарування, морфологічне дослідження.*

Author's contribution

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