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Changes in the Mineral Composition of Dental Hard Tissue Under Demineralizing Conditions: In Vitro Study

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ABSTRACT

Background: In the structure of tooth enamel, a high proportion of mineral components plays a crucial role in the protective functions of these structures. Moreover, enamel becomes inevitable for cariogenic microorganisms in high mineralization conditions. Mitigation of factors compromising enamel microstructures significantly alleviates the risks of decay. One of demineralization's most significant adverse outcomes is its contribution to caries formation. However, demineralization is a reversible process - organisms possess immune capabilities to restore mineral balance in enamel, a process referred to as remineralization.

Objectives: We aimed to scrutinize the mineral composition of tooth enamel using a scanning electron microscope.

Methods: For this purpose, we selected the extracted teeth from 16 individuals without any discernible pathology. Each tooth was sectioned into two parts, resulting in 32 experimental samples. Samples were divided into two groups: control and study groups.

The elemental composition of teeth in the control group was examined using JEOL's scanning electron microscope. While on the samples of the control group, we conducted a simulated process of caries using our modified in vitro method, known to induce enamel demineralization. After completing the experiments, we analyzed the distribution of microelements in the enamel using electron microscopy and compared the obtained data with the control group's results.

Results: As a result, we found that (i) the concentration of target elements (Ca, P) in the enamel increased approximately twofold compared to dentin's layers; (ii) the concentration of F increased almost sixfold in the enamel compared to its concentration in dentin; (iii) in the experimental caries model, the concentration of Ca and P in enamel (both in enamel and dentin) significantly decreased, while the concentration of F dropped to zero.

Conclusions: Objectively evaluating the results of conservative methods and comparing laboratory data and quantitative and qualitative changes in periodontal markers before and after treatment, it can be concluded that the most reliable modality is the integrated treatment of periodontitis.

Keywords: Demineralization; experimental caries; mineral composition of tooth enamel; remineralization.

INTRODUCTION

In the human organism, various tissues - enamel, dentine, cementum, and pulp - represent natural "hybrids" of organic and inorganic substances. Among them, pulp, dentine, and cementum are specialized, intermediate organic synthesis products, with their organic part predominantly represented by type I collagen. However, unlike dentine and cementum, 90% of the organic fraction is non-collagenous - amelogenins in the mineralized part of the pulp.[1-3]

The distribution of mineral components in the structure of these tissues significantly affects their supporting-mechanical function. The highest concentration of non-organic filling is found in the pulp (95%), comparatively less in dentine (70%), and the lowest in cementum (45%) (Fig.1 and Fig.2). The significant difference between organic and non-organic parts determines the physical properties of enamel tissues - the hardness of dentin and the elasticity of its adjacent dentine.[4]

The upper layer of enamel consists of hydroxyapatite (HA) crystals. Hydroxyapatite is a crystal form of calcium (CA^{++}), hydroxyl (OH), and phosphate ($PO43^{-}$) ions. Tightly packed crystals form enamel prisms, which are the structural units of enamel.[5-7] The upper layer of enamel is denser and more mineralized than its deeper layer, dentin. The structural integrity of enamel prisms, arranged in the enamel rods, also contributes to the contact stability of the structures in the cusps of teeth.[4,8,9]

The hardness of the upper layer of enamel is primarily due to the high concentration of phosphate ($PO43^{-}$) and calcium (CA^{++}) ions. The dentin adjacent to the enamel is less mineralized due to lower concentrations of phosphate and calcium but higher concentrations of magnesium (Mg^{++}), sodium (Na^{+}), and potassium (K^{+}) ions.[10,11]

Mineralized enamel is difficult for microorganisms to penetrate. Although the pellicle, mucin contained in saliva, pH of the oral cavity, and specific factors of oral cavity protection create conditions hindering cariogenic

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microorganisms, the most reliable guarantee of tooth protection is high enamel mineralization.[4,12]

FIGURE 1. Distribution of water, organic, and inorganic components in enamel by weight (A) and volume (B)

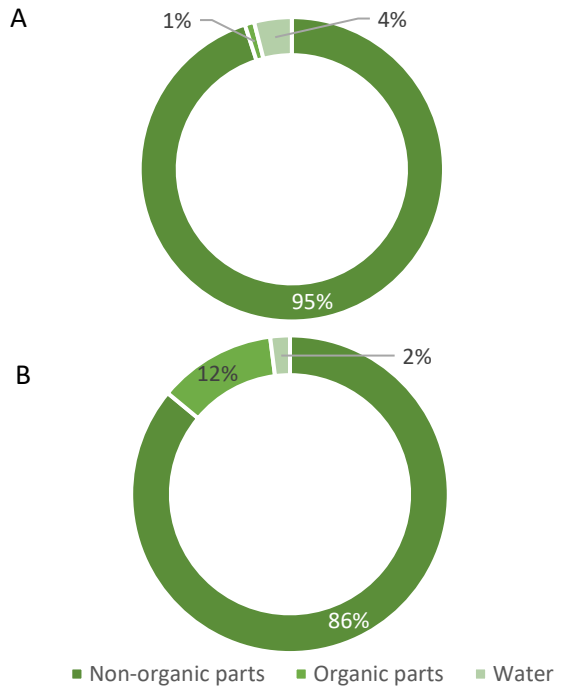
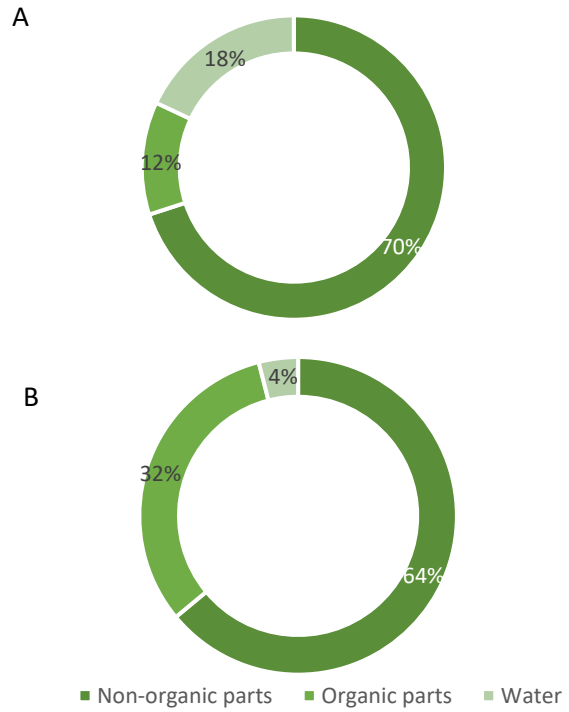


FIGURE 2. Distribution of water, organic, and inorganic components in dentin by weight (A) and volume (B)



The weakening of the factors mentioned above creates conditions for easy penetration of microorganisms into the internal structures of the tooth. One of the most critical negative manifestations of enamel penetration is its demineralization.

During demineralization, the mineral composition of hydroxyapatites is broken by the action of acids produced by microorganisms in dental plaque. Apatite crystals are most stable when the pH of the mouth is 7.5. When the pH drops below 5.5, the crystals begin to break, and the inter-crystal spaces expand. As a result, the surface of the enamel becomes soft and porous, which leads to caries. It should not be understood as if enamel demineralization is an irreversible process that must necessarily end with the formation of a carious defect. The body's immune forces can ensure the regression of the imbalance in the structure of the teeth and the restoration of balance. This process is called biomineralization.[1,13-15]

Biomineralization is a dynamic, complex, continuous process that regulates the deposition of inorganic nanocrystals in the organic matrix of living organisms. As a result, unique hybrid biological tissues (enamel, dentin, cementum, and bone) are formed.[1] As we mentioned earlier, this process takes place throughout life. However, it may reverse and develop demineralization under certain conditions and external factors.[16,17] Teeth and bones face these dangers throughout life. The high risk of demineralization is caused by the peculiarities of the anatomical arrangement and structure of the teeth - relief surface, natural grooves, hollows, and others. In addition, teeth are constantly affected by some components of food and drink and oral microorganisms.[18,19] However, it should also be noted that due to the above reasons, teeth develop a high resistance to demineralization, which bone does not have.[1]

The processes mentioned above are the basis for maintaining the health of the cool tissues of the tooth. Any deviation from the norm stimulates the mobilization of the body's protective forces and reverses the process.

However, such a factor as the delay time must also be considered. In other words, the long-term effect (delay) of undesirable factors on the tooth's surface. In the latter conditions, the demineralization-remineralization process, unfortunately, tends to favor the former.[12,20]

Unsurprisingly, the search for conditions and methods stimulating or inhibiting remineralization has always been a subject of special attention for dentists.

Maintaining the integrity of the tooth surface and selecting the best among the many pharmacological agents available on the market to achieve this goal will only be possible if the doctor (researcher) knows the essence and anatomy of the demineralization process and why demineralization is based on the change of mineral components in the structure of enamel and dentin. It is the

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study of the dynamics of these microelements that sheds light on the problem.[21]

The above determined the purpose of our research: to determine the composition of microelements (Ca, P, and F) in the enamel and dentin of undamaged human teeth and to determine their concentration changes during demineralization caused by experimental conditions. In order to achieve this goal, we set the following tasks:

- To determine the distribution of microelements in the enamel and dentine of extracted human teeth by X-ray spectral method;
- Modeling the carious process in vitro on extracted teeth and studying target microelements in enamel and dentin under these conditions.

METHODS

To determine the concentration of target microelements and to study their changes, we selected 16 extracted, intact human teeth. The indication for tooth extraction was their trauma, orthodontist's, or periodontist's decision. The age of the patients ranged from 16 to 60 years. The procedure for storing extracted teeth was carried out by the national protocol of the state standard for clinical condition management, "Prevention of infection when handling extracted human teeth, biopsies of tissues surrounding the tooth, and operative material" (2020. 21. 02, #01-282/o). We studied the concentration of microelements Ca, P, F in the following areas of enamel and dentin: in enamel (zone 1), and in dentin - near the border of enamel (zone 2), and in the parapulpal area (zone 3).

Each tooth was cut longitudinally with a separating disc and divided into two parts (halves). In this way, we got 32 experimental specimens. One-half of the separated teeth (16 samples) were used to study microelements in enamel and dentin, and they formed the control group. We performed acid etching on them (with 35% phosphoric acid - "Ultra-Etch"), fully following the steps provided by the protocol. After washing and drying the teeth of the control group, their elemental composition was examined. The research was carried out with a JEOL scanning electron microscope.

It should be noted that the atoms of the elements in any material (including teeth) only have their characteristic radiation, wavelength, and intensity. This principle is the basis of X-ray spectral analysis methods of materials, which makes it possible to determine the elemental composition of the substance.

The distance from the electron output tube to the surface of the experimental tooth sample was 15 mm. In order to reduce the surface charge and cover the samples with 10 nm of platinum, they were made with a thick layer, for which we used the vacuum coating equipment of the Japanese company JEOL. The software of the analyzer allows us to receive the result of the analysis in the form of a Word document, where the X-ray radiation spectra of the areas marked on the research object (both in mass and atomic percentages), tables, elements identified in the target areas and their concentration were reflected.

In the second stage of our research, we modeled the caries process on the second halves of the separated teeth (16 samples) using our modified method based on the demineralization of cool tissues. These samples constituted the study group. Modeling of experimental caries is based on the cyclical change of PH of the environment affecting the tooth. For this purpose, we placed the samples for 6 hours (11:00-17:00) in an acetone buffer (5 ml of a solution with a pH of 3.5 for each sample). During the remaining 18 hours (17:00-11:00), we left the samples in a solution with a neutral pH (Tris buffer). During the weekend, we soaked the samples in a neutral solution. On the sixth day of the experiment, we changed the solutions and continued the regimen for another five days. It took ten days to model the demineralization process in the study group samples.

After the end of the experiment, we studied the content of microelements in the teeth of the research group by scanning electron microscopic examination. We compared the data we obtained with the indicators of the control group. We have not changed the numbering of the samples to avoid errors. The numerical data in the tables were statistically processed, showing the mean square deviation.

RESULTS AND DISCUSSION

The study showed that the highest calcium concentration was found in the hard tissues of the teeth of the control group (18.33). Phosphorus (9.5) was in second place, and fluorine was found with the lowest concentration (0.22) (Tab.1).

TABLE 1. The content of microelements (Ca, P, F) in the hard tissues of the teeth of the control group: enamel, dentin-enamel border, parapulpal dentin

Trace elements	Layer	Concentration (%)	t	p
Ca	Enamel	28.2±2.7	1.15	>0.05
	Dentin-enamel	13.8±4.7		
	Parapulpal dentin	12.99±1.75		
P	Enamel	13.9±0.6	2.77	<0.01
	Dentin-enamel	6.8±2.1		
	Parapulpal dentin	6.5±0.74		
F	Enamel	0.51±0.3	2.62	<0.01
	Dentin-enamel	0.07±0.03		
	Parapulpal dentin	0.09±0.04		

Research has shown that the enamel layer is two times richer in calcium than dentin. In particular, calcium in enamel was found to be 28.2, and in both layers of dentine together - 13.39. A similar trend occurred in the case of phosphorus. In particular, its concentration in the enamel layer was 13.9; in the two dentin layers, it was 6.65.

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Studying the concentration of trace element fluorine was of particular interest to us. As the study showed, fluoride concentration in enamel and dentin was distributed differently than the same indicator in the case of calcium and phosphorus. In particular, the fluoride concentration in the enamel layer was six times higher than the same indicator in both dentin layers (0.51 and 0.08, respectively).

The results of the second stage of the study revealed that the concentration of calcium in the dentine layer significantly decreased in the experimental caries conditions compared to the same level in the enamel layer. In particular, if the average rate of calcium concentration in dentin in the control group was 13.3 (Tab.2), in the control group, the same rate decreased almost three times and equaled 4.9. The study of microelement phosphorus in the study group revealed the same trend. Phosphorus concentration at the dentin-enamel border and parapulpal zone decreased 2.7 times compared to the control group.

TABLE 2 The content of microelements (Ca, P, F) in the hard tissues of the teeth after demineralization

Trace elements	Layer	Concentration (%)	t	p
Ca	Enamel	25.64±3.1	0.38	>0.05
	Dentin-enamel	6.79±3.0		
	Parapulpal dentin	3.2±2.5		
P	Enamel	12.6±5.2		
	Dentin-enamel	3.58±1.2		
	Parapulpal dentin	1.72±1,32		
F	Enamel	0	-	-
	Dentin-enamel	0		
	Parapulpal dentin	0		

We obtained a dramatic result when studying the trace element fluorine. In the conditions of experimental caries (during demineralization of cool tissues of the tooth), it could not be identified in any of the experimental samples (there were 16 of them).

CONCLUSIONS

The results of our research allowed us to make the following conclusions:

- Calcium content in tooth enamel is two times higher than the same indicators in dentine;
- Phosphorus concentration in tooth dentin is almost two times less than the same trace element in enamel;
- The concentration of fluoride in the enamel layer is six times higher than the same indicator in the dentin;
- After demineralization, the concentration of calcium and phosphorus in dentine decreased by about three times and in enamel by only 1.1 times;
- After the experimental caries, the fluoride concentration in the tooth's hard tissues was equal to zero.

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