

# THE PROCESS OF FORMATION OF TOOTH ENAMEL BY THE METHOD OF ELEMENTAL DISPERSION SPECTRAL ANALYSIS

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## ABSTRACT

The purpose of this work was to determine the degree of maturity of the enamel of permanent teeth and to study the content of trace elements in its surface layers using elemental dispersion spectral analysis. The material for the study was the permanent third molars of children from 16 to 18 years old. 51 intact permanent third molars that had not erupted were examined, which was at the stage of root growth in length. Analyzing the atomic chemical composition of tooth enamel, we found out that oxygen, sodium, chlorine, calcium, phosphorus were detected in 100% of the samples. Elements, Mg<sup>2+</sup> was in 63.89% of samples, F<sup>-</sup> – in 30.56%, C<sup>4+</sup> – in 13.89%, S<sup>2-</sup> – in 8.33% of samples. The content of Ca<sup>2+</sup> was 18.87±6.28 (atom. %), the content of P<sup>5+</sup> is 13.45±3.44 (atom. %). The enamel of teeth that have not erupted or only erupted is immature. At the same time, it was found that the strength of dental tissues is influenced not only by the optimal ratio of basic trace elements, such as calcium and phosphorus, but also by an increase in the amount of magnesium, sodium, potassium, silicon and a decrease in sulfur and chlorine content \*3, 5+. Thus, this paper shows the advantages of using elemental dispersion spectral analysis in the study of enamel mineralization in the dynamics of the process of tooth formation.

**Key words:** Tooth enamel, Elemental dispersion spectroscopy, X-ray, Third molars.

## Introduction

Mineralized and non-mineralized tissues are isolated in the tooth. The first include enamel, dentin and cement. In general, there are normally four types of mineralized tissues in the human body: enamel, dentin, cement and bone, which differ in chemical composition and origin [1]. The last three originate from mesoderm stem cells, whereas enamel is a derivative of ectoderm [2]. Their chemical composition is dominated by inorganic components, and organic compounds and water are also present [3, 4].

Resistance to dental caries is ensured by the correct laying and formation of the rudiments of teeth, the physiological development of the hard tissues of the tooth \*4, 12+ [5, 6]. One of the conditions for the resistance of teeth to caries is the formation of a full-fledged enamel structure, which begins with the formation of a protein matrix and ends with the mineralization of enamel [7]. Mineralization is the process of saturation of tooth enamel with macro- and microelements, as a result of which the resistance of enamel to cariesogenic factors increases [8]. Mineralization occurs within 1.5-2 years after teething. Calcium, fluorine, phosphorus and magnesium are used in the mineralization process. The natural source of Ca is oral fluid. The highest degree of mineralization is on the tubercles of the teeth, the lowest is in the neck of the tooth. [9]. The usefulness of

mineralization is ensured by a properly formed protein matrix \*6, 8, 15+ [10]. In the case of an incomplete mineralization process, pathologies arise, mainly associated with carious diseases [11-13]. To diagnose such disorders, it is possible to study the dynamics of the elemental composition of teeth. Changes in the elemental composition of teeth can reveal metabolic disorders in the process of tooth development after eruption \*13, 14+. Therefore, the study of the chemical composition of the surface layers of the enamel of these teeth is relevant [14-17]. One of the universal methods of solving this problem can be the use of diagnostics based on the method of elemental dispersion spectral (EDS) analysis [18-21]. The purpose of this work was to determine the degree of maturity of the enamel of permanent teeth and to study the content of trace elements in its surface layers using EDS analysis.

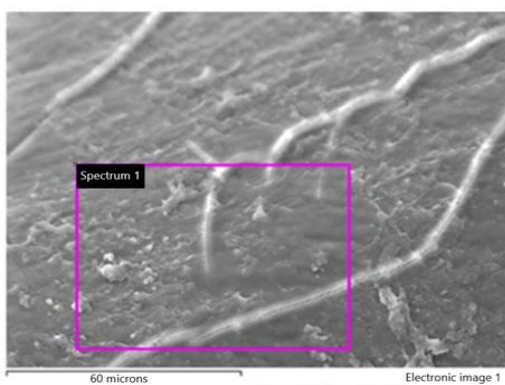
## Materials and Methods

EDS analysis of teeth was carried out using an AIS 2300 C scanning electron microscope (Seron Technology, South Korea). To determine the trace element composition, the method of electron dispersion X-ray spectroscopy is indicative and accurate. The material for the study was the permanent third molars of children from 16 to 18 years old. 51 non-erupted intact permanent third molars were examined, which was at the stage of root growth in length.

The criteria for choosing permanent third molars were: similarity of developmental stages and morphology with permanent first molars in 6-year-old children, the possibility of obtaining material for research, namely, removed teeth according to orthodontic indications. The removed teeth were washed with distilled water for three minutes. All samples were stored in tightly closed tanks (10% streptomycin solution) at a temperature of (+2... + 4) °C for two days.

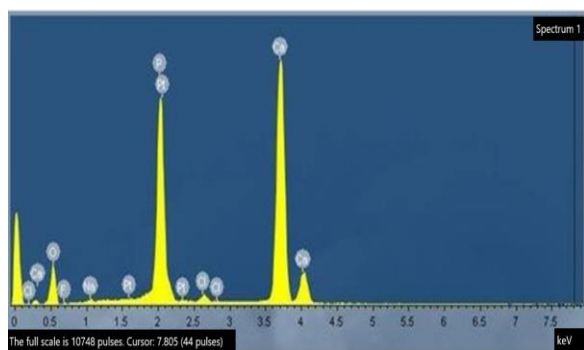
## Results and Discussion

Two days later, the test samples were prepared for the determination of trace element composition by electron dispersion X-ray spectroscopy. The content of chemical elements in the surface layer of permanent tooth enamel was determined in the equator zone, tubercle and neck. The size of the sections of the enamel surface under study ranged from 50x50 µm to 250x250 µm (**Figure 1**).



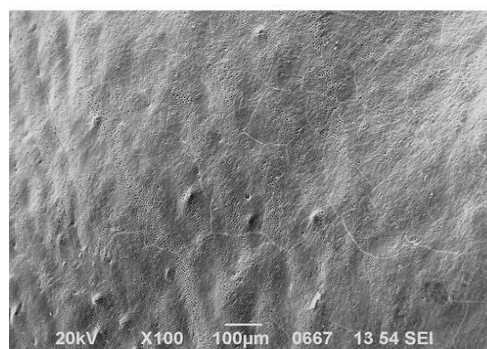
**Figure 1.** The study site of immature permanent tooth enamel in an X-ray dispersion spectral analyzer

The content of chemical elements in the surface layer of permanent tooth enamel and the initial mineralization level of each sample were determined using an X-ray characteristic spectrum (**Figure 2**).

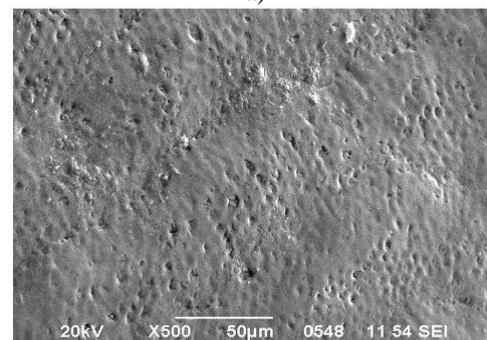


**Figure 2.** X-ray characteristic spectrum of the surface layer of permanent tooth enamel

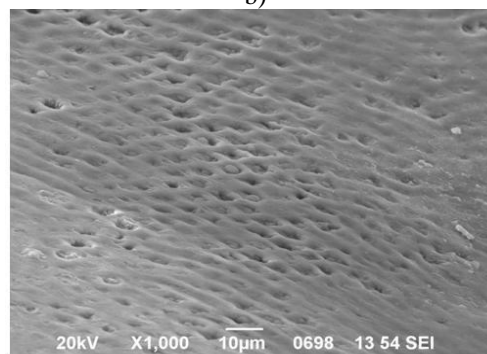
When examining the surface of the enamel of teeth, optimal magnification modes were determined (×100, ×500, ×1000, ×3000) (**Figures 3a-3d**).



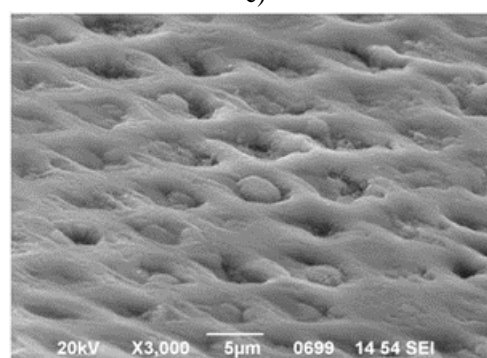
a)



b)



c)



d)

**Figure 3.** Immature permanent tooth enamel: ×100(a), ×500 (b), ×1000 (c), ×3000 (d)

When studying the chemical composition of tooth enamel by the EDS method, similar indicators of the weight content of trace elements (%) were determined in our studies:  $\text{Ca}^{2+}=32.67\pm 8.07$ ;  $\text{P}^{5+}=17.84\pm 4.10$ ;  $\text{Na}^{+}=0.84\pm 0.23$ ;  $\text{Mg}^{2+}=0.15\pm 0.11$  (**Table 1**). In fact, we obtained results in which weight calcium and magnesium tended to decrease,

and phosphorus and sodium were within the normal range \*9+.

**Table 1.** The results of the study of the chemical composition of the enamel of permanent teeth by the EDS method

Elemental composition	Chemical element	Number of samples studied	The number of samples having a chemical element		Quantitative composition of samples, %
			Absolute value	%	
Atomic	O	36	36	100	61.48±9.68
	F	36	11	30.56	0.97±0.40
	Na	36	36	100	0.85±0.34
	Cl	36	36	100	0.58±0.36
	Ca	36	36	100	18.87±6.28
	Ca/P	36	23	63.89	0.14±0.10
	Mg	36	3	8.33	0.76±0.12
	C	36	5	13.89	31.08±16.91
Weight	O	36	36	100	44.05 ±8.77
	F	36	11	30.56	0.83±0.38
	Na	36	36	100	0.84±0.23
	Cl	36	36	100	0.89±0.64
	Ca	36	36	100	32.67±8.07
	Mg	36	3	8.33	1.14±0.29
	C	36	5	13.89	21.33±13.68

Analyzing the atomic chemical composition of tooth enamel, we found out that oxygen, sodium, chlorine, calcium, phosphorus were detected in 100% of the samples. Mg<sup>2+</sup> elements were found in 63.89% of samples, F<sup>-</sup> in 30.56%, C<sup>+</sup> in 13.89%, S<sup>2-</sup> in 8.33% of samples. The content of Ca<sup>2+</sup> was 18.87±6.28 (atom. %), the content of P<sup>5+</sup> is 13.45±3.44 (atom. %). The output level of mineralization by the atomic (%) Ca / P ratio was 1.40 and was closer to the lower limit (1.33), after which irreversible changes in the structure of enamel \*2+ are observed. A lower Ca/P coefficient was found than the average values for human tooth enamel \*1+. This shows that the enamel of teeth that have not erupted or only erupted is immature.

At the same time, it was found that the strength of dental tissues is influenced not only by the optimal ratio of basic trace elements, such as calcium and phosphorus, but also by an increase in the amount of magnesium, sodium, potassium, silicon and a decrease in sulfur and chlorine content \*3, 5 +.

## Conclusion

The atomic chemical composition of the tooth enamel surface made it possible to investigate electron dispersion spectral analysis. We found out that 100% of the samples contained such chemical elements as O<sup>2+</sup>, Na<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, P<sup>5+</sup>;

Mg<sup>2+</sup> was contained in 63.9% of the samples, F<sup>-</sup> in 30.6%, C<sup>+</sup> in 13.9%, S<sup>2-</sup> in 8.3% of the samples. The content of Ca<sup>2+</sup> was 18.8±6.28 (atom. %). The content of P<sup>5+</sup> is 13.45±3.44 (atom. %). The output level of mineralization by the atomic (%) Ca/P ratio was 1.40 and was closer to the lower limit (1.33). A lower Ca/P coefficient was revealed than the average values for human tooth enamel. This shows that the enamel of the teeth that had just erupted was immature. The data obtained by us coincide with the results of studying the amount of fluorine, calcium and phosphorus in the enamel by X-ray photoelectron spectroscopy, which established an insufficient level of mineralization of the enamel of permanent teeth after eruption \* 10+. The presence of plaque on the teeth and the effect of other cariesogenic factors, especially during this period, are very dangerous, which indicates the need to develop and carry out preventive measures, in particular exogenous ones, aimed at accelerated mineralization of immature enamel of permanent teeth.

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**Ethics statement:** None

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