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Unveiling the Formation of Tooth Enamel Using Elemental Dispersion Spectral Techniques

Dayana Kazbekovna Kushkhova[1], Arnella Alikovna Kozyreva[1], David Gennadievich Valiev[1]*, Adelina Rafikovna Kallagova[1], Selita Salmanovna Elzhurkaeva[2], Karina Admovna Kakaeva[3]

[1]Department of Therapy, Faculty of Dentistry of the North Ossetian State University named after K. L. Khetagurov, Vladikavkaz, Republic of North Ossetia-Alania, Russia.
[2]Department of Therapy, Faculty of Dentistry of the North Ossetian State Medical Academy, Vladikavkaz, Republic of North Ossetia-Alania, Russia.
[3]Department of Therapy, Faculty of Medicine of the North Ossetian State Medical Academy, Vladikavkaz, Republic of North Ossetia-Alania, Russia.

*E-mail ✉ ruslankalmykov777@yandex.ru

ABSTRACT

This study aimed to investigate the maturity of enamel in permanent teeth and investigate the trace element composition in its surface layers through elemental dispersion spectral analysis. This research focused on third molars from individuals aged 16 to 18 years, specifically 51 intact molars that had not yet erupted but were in the root development phase. The atomic chemical analysis of tooth enamel showed that oxygen, chlorine, sodium, calcium, and phosphorus were found in all the samples. In addition, magnesium (Mg^{2+}) was present in 63.89% of the samples, fluoride (F^-) in 30.56%, carbonate (C^{4+}) in 13.89%, and sulfur (S^{2-}) in 8.33%. The average concentration of calcium (Ca^{2+}) was 18.87 ± 6.28 (atom. %), while phosphorus (P^{5+}) was 13.45 ± 3.44 (atom. %). Enamel from unerupted or partially erupted teeth was found to be in an immature state. The study also showed that the integrity of dental tissues is affected not just by the balanced levels of essential elements such as calcium and phosphorus, but also by increasing the concentration of potassium, magnesium, sodium, and silicon, with a decrease in chlorine and sulfur. The results emphasize the usefulness of elemental dispersion spectral analysis in monitoring the mineralization process of enamel during tooth development.

Keywords: Elemental dispersion spectroscopy, Tooth enamel, X-ray, Third molars

Introduction

Teeth consist of both non-mineralized and mineralized tissues, with dentin, enamel, and cement being the primary mineralized components. In the human body, 4 types of mineralized tissues exist: dentin, enamel, cement, and bone, each differing in their chemical makeup and origin [1-3]. While the latter three arise from mesodermal stem cells, enamel originates from ectodermal cells. The chemical composition of these tissues is primarily dominated by inorganic substances, with some water and organic compounds present as well [4, 5].

The resistance of teeth to caries is linked to the proper formation and development of their hard tissues. One essential factor in this resistance is the proper development of enamel, which starts with forming the protein matrix and concludes with the mineralization of the enamel [6-9]. Mineralization is a critical process where the enamel is enriched with macro- and microelements, strengthening its resistance to caries-inducing factors. This process typically occurs within 1.5-2 years after the eruption of the teeth, involving

elements such as calcium, fluoride, phosphorus, and magnesium [10, 11]. Calcium, derived from the oral fluid, plays a key role in this process, and the highest mineralization occurs at the peaks of the teeth, while the lowest is found at the neck of the tooth [12-14].

Proper mineralization depends on a well-formed protein matrix. When this process is incomplete, dental pathologies, primarily caries, can develop [15, 16]. To diagnose these issues, it is necessary to monitor changes in the elemental composition of the teeth. By studying the dynamics of the elemental makeup, we can identify metabolic disruptions that may arise during tooth development, even after eruption. Therefore, investigating the chemical composition of enamel surface layers is crucial, and elemental dispersion spectral (EDS) analysis serves as a reliable diagnostic tool for this purpose [17-21]. This study aims to assess the maturity of permanent tooth enamel and analyze the trace element content in its surface layers using EDS analysis.

Materials and Methods

The elemental composition of the teeth was analyzed using an AIS 2300 C scanning electron microscope (Seron Technology, South Korea). For the identification of trace elements, electron dispersion X-ray spectroscopy was employed, as it provides precise and reliable results. The study focused on the permanent third molars of children aged sixteen to eighteen years. A total of 51 unerupted, intact third molars, still in the root growth stage, were selected for examination. The criteria for selecting these teeth included their developmental and morphological similarity to the permanent first molars of six-year-old children, as well as the availability of suitable samples, which were extracted for orthodontic reasons. Following extraction, the teeth were rinsed in distilled water for 3 minutes. The samples were then stored in sealed containers containing a 10 percent streptomycin solution at a temperature range of (+2 ... +4) °C for two days to preserve their condition.

Results and Discussion

After two days, the samples were readied for trace element analysis using electron dispersion X-ray spectroscopy. The chemical element concentrations in the surface layer of permanent tooth enamel were measured in various regions, including the tubercle, equator zone, and neck. The enamel surface sections analyzed varied in size, ranging from 50 x 50 µm to 250x250 µm **(Figure 1)**.
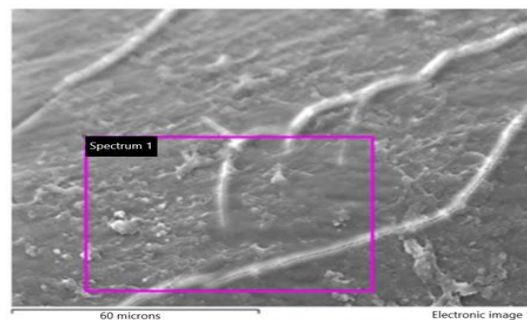

Figure 1. The study site of immature permanent tooth enamel in an X-ray dispersion spectral analyzer

The chemical element composition in the surface layer of permanent tooth enamel, along with the initial mineralization level of each sample, was assessed using an X-ray characteristic spectrum **(Figure 2)**.
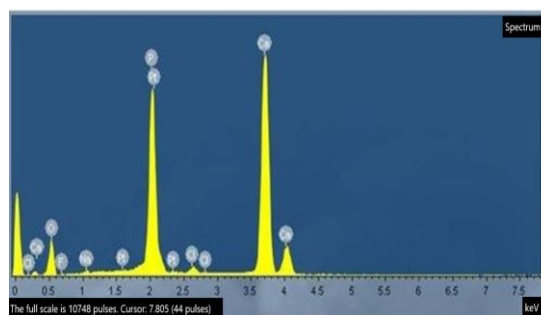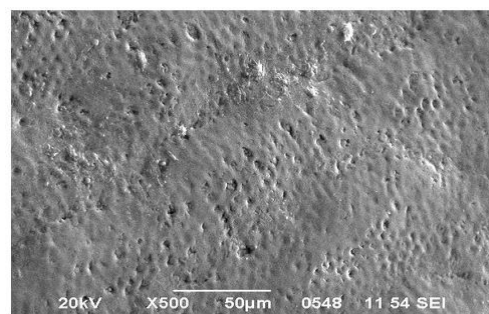

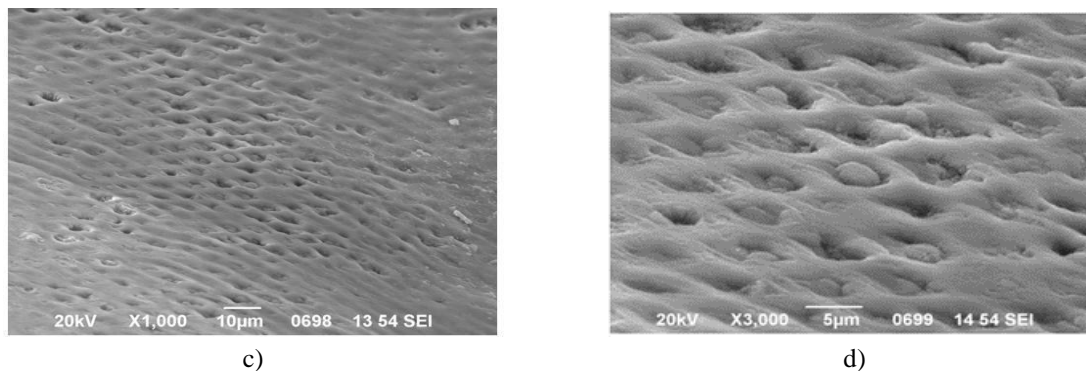**Figure 2.** X-ray characteristic spectrum of the surface layer of permanent tooth enamel

During the examination of the enamel surface, the most suitable magnification levels were established (×100, ×500, ×1000, ×3000) **(Figures 3a-3d)**.

a)

b)

c)

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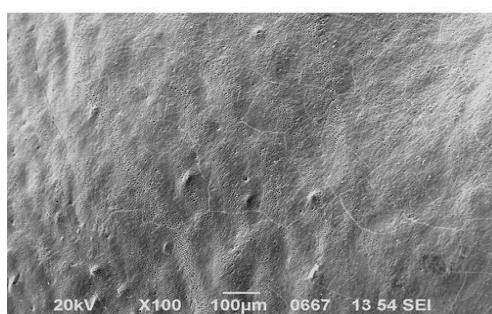
Figure 3. Immature permanent tooth enamel: ×100 (a), ×500 (b), ×1000 (c), and ×3000 (d)

In our analysis of tooth enamel using the EDS technique, we observed consistent values for the weight percentage of trace elements: Ca^{2+} = 32.67 ± 8.07; P^{5+} = 17.84 ± 4.10; Na^+ = 0.84 ± 0.23; Mg^{2+} = 0.15 ± 0.11 **(Table 1)**. The results indicated a slight reduction in the levels of calcium and magnesium, while phosphorus and sodium remained within expected normal ranges *9+.

Table 1. The chemical composition of permanent tooth enamel analyzed by the EDS method

Chemical element	Elemental composition	Number of samples studied	Number of samples containing element	Quantitative composition of samples (%)	Atomic composition (%)	Weight composition (%)
O	Oxygen	36	36	100	61.48 ± 9.68	44.05 ± 8.77
F	Fluorine	36	11	30.56	0.97 ± 0.40	0.83 ± 0.38
Na	Sodium	36	36	100	0.85 ± 0.34	0.84 ± 0.23
Cl	Chlorine	36	36	100	0.58 ± 0.36	0.89 ± 0.64
Ca	Calcium	36	36	100	18.87 ± 6.28	32.67 ± 8.07
Ca/P	Calcium/ Phosphorus	36	23	63.89	0.14 ± 0.10	-
Mg	Magnesium	36	3	8.33	0.76 ± 0.12	1.14 ± 0.29
C	Carbon	36	5	13.89	31.08 ± 16.91	21.33 ± 13.68

Upon analyzing the atomic composition of tooth enamel, the researchers detected oxygen, chlorine, sodium, calcium, and phosphorus in all samples. Mg^{2+} was present in 63.89% of the samples, while F^- appeared in 30.56%, C^{4-} in 13.89%, and S^{2-} in 8.33%. The Ca^{2+} content was 18.87 ± 6.28 (atom. %), and P^{5+} was 13.45 ± 3.44 (atom. %). The Ca/P ratio for mineralization was 1.40, which is closer to the lower threshold (1.33), a point after which irreversible enamel structure changes are typically observed *2+. The Ca/P ratio found in this study was lower than the average values for tooth enamel *1+, suggesting that the enamel of teeth that have not yet erupted or have just erupted is still immature. Additionally, the strength of dental tissues depends not only on the balanced ratio of primary trace elements like calcium and phosphorus but also on increased levels of magnesium, potassium, sodium, and silicon, while the content of sulfur and chlorine decreases *3, 5+.

Conclusion

The electron dispersion spectral analysis of tooth enamel allowed us to assess the atomic chemical composition of its surface. Our findings revealed that all samples contained oxygen (O^{2+}), sodium (Na^+), chlorine (Cl^-), calcium (Ca^{2+}), and phosphorus (P^{5+}). Additionally, magnesium (Mg^{2+}) was present in 63.9% of the samples, fluoride (F^-) in 30.6%, carbonate (C^{4-}) in 13.9%, and sulfate (S^{2-}) in 8.3%. The calcium content was measured at 18.8 ± 6.28 (atom. %), while phosphorus was at 13.45 ± 3.44 (atom. %). The mineralization level, as determined by the atomic Ca/P ratio, was 1.40, which is closer to the lower threshold of 1.33. This ratio was lower than the typical values found in human tooth enamel, indicating that the enamel of newly erupted teeth is still immature. Our results align with those obtained from X-ray photoelectron spectroscopy, which identified insufficient mineralization in the enamel of permanent

teeth post-eruption *10+. The presence of plaque and the impact of other caries-inducing factors, especially during this developmental phase, highlight the importance of implementing preventive strategies, particularly those that promote the accelerated mineralization of immature enamel in permanent teeth.

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