U.P.B. Sci. Bull., Series B, Vol. 85, Iss. 3, 2023 ISSN 1454-2331

RESEARCH ON THE VARIATION OF THE CHEMICAL COMPOSITION BY SEM IN THE CASE OF A SET OF TEETH FOR A POPULATION FROM THE AREA OF THE CITY OF BUCHAREST

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The problem of the mineralization of bone structures in the context of the variations of their chemical elements depending on age represents an intense occupation at the present time in medicine and pharmacy, being insisted on by society. On the pharmaceutical market, there are currently extremely many offers regarding food supplements with different contents in certain chemical elements that are predominantly found in bone structures. Since teeth also represent structures of this type that can be accessed more easily, the current research followed the evaluation of the chemical composition of teeth in their two representative areas: dentine and enamel. Based on this evaluation, the variation graphs of the main chemical elements determined by SEM microscopy were made. The conclusion of this analysis was that the age of 40 years represents a turning point in the variation of chemical elements such as O_2, C, Si, Mg, P and Na.

Keywords: teeth, chemical, composition, variation, time.

1. Introduction

In nature there are many essential elements that have a strong impact on dentin and bone tissue. As the main inorganic component of dental enamel and bones [1,2], hydroxyapatite (Hap) is considered the most important supporting substance. The proportion of hydroxyapatite in teeth is much higher than in bones, where we have approximately 65% mineral, the rest being made up of collagen and osteoblasts. Due to this difference, teeth are much harder than bones. Looking at the chemical composition of the teeth, it can be observed that enamel [8,9,10,11], the very resistant region that covers the outside of the tooth, contains over 95% inorganic substances, 2,3% water and 1,7%. organic substances.

Cement has approximately 46% inorganic substances, 32% water, 22% organic substances and dentin [3,5,6,7], the harder middle region, contains 69-70% inorganic substances, water 13,2 and 17,8 organic substances. Dental pulp, the soft central area,

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has 68% water, 28% organic substances and 3,9-4% inorganic substances. Major (e.g. Ca, P), minor (e.g. Cl, Mg, Na) and trace elements (Cd, Zn) [4,12] are incorporated within teeth. Depending on changes that occurred as a result of an individual s exposure and the function of the teeth, the distribution of these elements inside the teeth varies.

Knowing the chemical composition of the teeth as well as any bony structure of the human body is a priority given their importance for the good morpho-functional functioning of the human body. For the study of the state of mineralization of the teeth, analyzes were made for 32 teeth for which the chemical composition was determined both in the area of the dentine and the enamel. The teeth were randomly selected knowing only the age of their owners. it was desired to use as few input data as possible so that the results provide a general answer as far as the chemical composition is concerned, without other influences such as sex, type of food, place of work, previous treatments. All these elements influence the structure of each tooth and under these conditions, a segregation of the results and their interpretation would be difficult.

For the presented research a SEM microscope was used to determine the chemical composition in the selected area presented in the figures. Scanning electron microscopy (SEM) is a rapid and convenient method for qualitatively analyzing the surface morphology of enamel and dentine specimens. All the probes preparation, SEM microscopy and chemical analyzes were performed in the Laboratories of the University POLITEHNICA from Bucharest.

2. About teeth structure

Teeth are important biological structures in the field of biomineralization. The mineral tissue of a tooth consists of hydroxyapatite (Hap) crystals, $Ca_{10}(PO_4)_6(OH)_2$. The inorganic content of dental hard tissues has been shown to consist of not only calcium (Ca), phosphorus (P), and oxygen (O), as indicated by the formula of calcium Hap [14], but also carbon (C), magnesium (Mg), sodium (Na), as well as large trace elements [15,16]. The structure of life consists, in many studies, in a proportion of almost 96% of nitrogen, hydrogen, carbon and several other chemical elements. Of these, around 50% of the elements can be measured using systems known in the scientific world. Physiological activities in mammals and humans use around 23 elements, of which 11 are trace elements (TE). These chemical elements are called transition elements, namely chromium, manganese, iron, copper, zinc, vanadium, as well as non-metallic chemical elements such as iodine, fluorine, selenium. TE are called micronutrients because they are needed in very small amounts, about 100 mg every day, unlike magnesium, calcium, sodium, potassium, chlorine or magnesium. All these elements play a very important role in health and the lack or higher amounts can lead to critical health problems. Inside part of the tooth, dentin represents the structure where the mass between the pulp area and enamel, which is made up of approximately 69% of inorganic chemical elements (hydroxyapatite and trace elements), approximately 18%

of organic chemical elements such as collagen, osteonectin, osteonectin-like protein GLA dentin or dentine matrix protein and approximately 13% water. Around 40% of elements with concentrations of 1000 ppm such as aluminum, zinc, iron, barium, lead at 100 ppm such as nickel, selenium, silver, lithium or niobium are found in dentin.

Enamel represents the structure of the tooth located on its exterior, which is characterized by a high hardness and aims to protect the inner volumes of the tooth, namely the dentin and the pulp. Its chemical composition consists of calcium hydroxy apatite which is approximately 95% followed by organic elements in approximately 2% and approximately 3% water. In a much smaller proportion inside the hydroxyapatite, which occupies 95%, there are elements such as magnesium, fluorine, cobalt, aluminum, selenium, lead, phosphorus, calcium or zinc. Trace elements appear in the structure of the tooth penetrating from the natural environment during and after the mineralization period of the tooth.

3. Chemical determination in teeth using SEM microscopy

In accordance with the chemical analyzes carried out in the last decades at the level of the teeth, the research presented followed the determination of this composition but viewed from another aspect. In this sense, for 32 teeth of randomly chosen patients from the Bucharest area, SEM microscopy was performed in the enamel and dentine area. For example, a few samples of the selected teeth were chosen, which were later analyzed. The only criterion considered in the study was the age of the patients. The other input data that could have been considered in the study, otherwise very important, such as gender, type of food, jobs were not selected here because the subsequent interpretations would have been very complicated and perhaps irrelevant. We only wanted to observe the variation of the chemical composition depending on the age, if this had happened. These were the initial considerations on which the authors agreed.

To exemplify the working method, Fig. 1 presents some samples of teeth prepared to be later analyzed by Scanning Electron Microscopy (SEM) microscopy analysis (morphological aspects of the obtained materials were studied, with a Quanta Inspect F50 microscope coupled with an X-ray Energy Dispersion Spectrometer (EDS) (Thermo Fisher, Eindhoven, The Netherlands).

For each of the studied samples, different areas on the surface of the enamel and respectively of the dentin were randomly chosen. Fig. 2 shows the chemical analysis for a tooth of a 17 year-old patient. As can be seen, the selected areas are clean, without cracks in their approach.

Fig. 1. Samples prepared for SEM microscopy

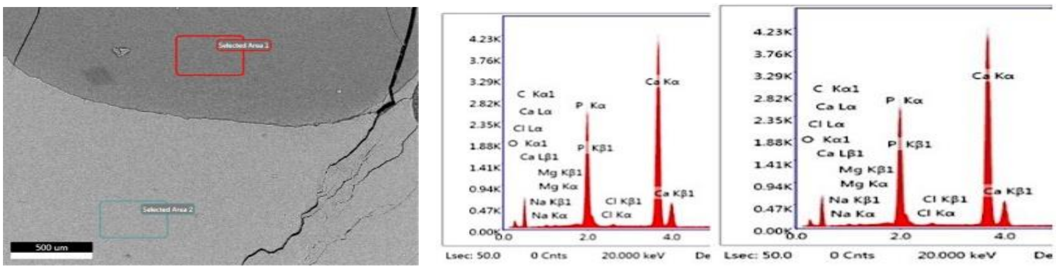

Fig. 2 Chemical analysis for a tooth of a 17 year-old patient

Fig. 3 presents the analysis for a tooth of a 28 year-old patient. And in this case, as can be seen, the selected areas are also clean, without cracks in their approach, although they can be seen surrounding the analyzed areas that means the dentine, area 1, and the enamel, area 2.

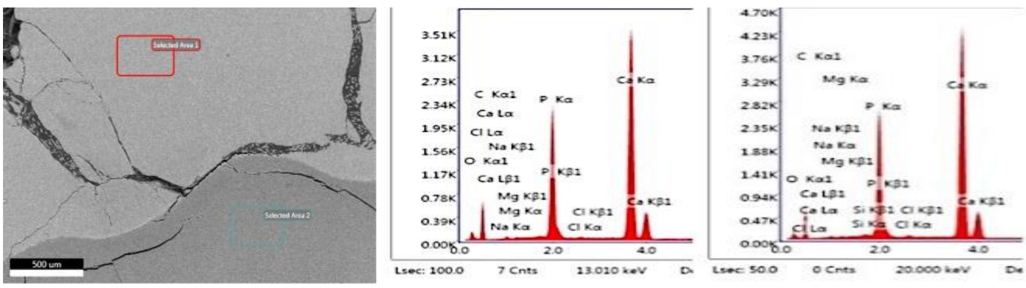

Fig. 3 Chemical analysis for a tooth of a 28 year-old patient

Fig. 4 illustrates the analysis of a tooth belonging to a 32-year-old patient. The enamel part, which is zone 2, appears to be narrower than usual, which could limit the available sample options. Nevertheless, it's easy to observe a clear boundary between the dentine and enamel.

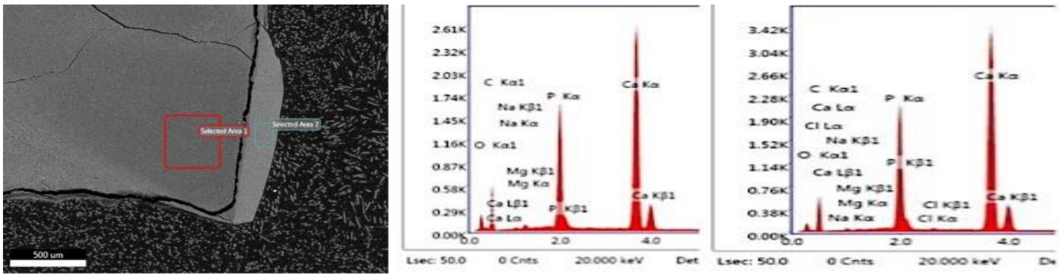

Fig. 4 Chemical analysis for a tooth of a 32 year-old patient

Fig. 5, permits the results of an analysis performed on a tooth belonging to a 34-year-old patient. The analysis revealed that the dentine part of the tooth, which is area 1, appears to be in good condition without any visible imperfections. However, there is an obvious crack present in both the dentine and enamel areas, which is noteworthy. It is also worth mentioning that the two areas are distinguishable from each other, and the analysis was conducted under favorable conditions.

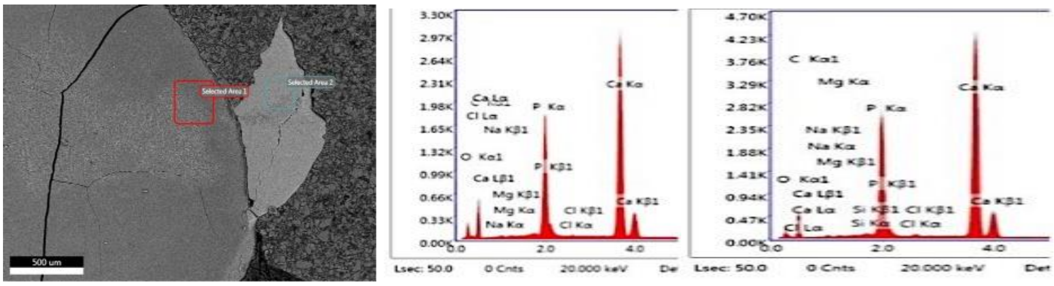

Fig. 5 Chemical analysis for a tooth of an 34 years-old patient

Regarding the sample of a 41-year-old patient, in the lower left, the dentin area can be seen, and on the right, in a darker color, the enamel area can be observed. A very distinct line of demarcation can also be observed between the two structures. The studied areas are clear, without microcracks or other impurities.

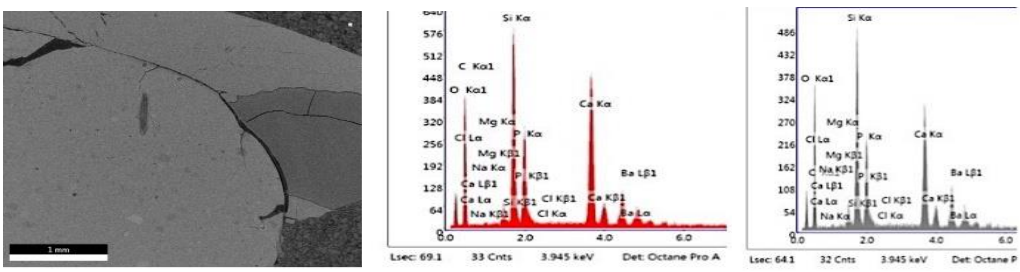

Fig. 6 Chemical analysis for a tooth of an 41 years-old patient

A sample from a 50-year-old patient is presented in Fig. 7. In area 1, an important crack can be observed, on the left side, while on the right side, area 2, a well-defined inclusion can be observed. Also, the interface between dentin and enamel is very well defined. The areas where the chemical compositions are determined are clean and specific to them.

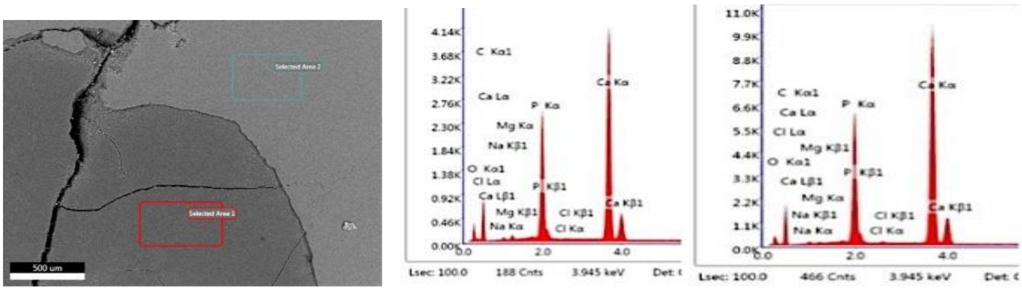

Fig. 7 Chemical analysis for a tooth of a 50 years-old patient

4. Discussions

For the interpretation of the results provided by the SEM analysis, the chemical compositions of the 32 analyzed samples were determined. In this sense, volume percentages of carbon, oxygen, phosphorus, magnesium, calcium and silicon were recorded. Fig. 8 presents the variation of these components in the dentin in function of the age of the patients from where the teeth were taken. At first observation, only a random variation of these chemical compositions can be observed. A closer study shows a correlation of them according to age, all having a certain characteristic with one exception. Around the age of 40, apart from the chemical content in carbon, all other chemical elements reach a minimum value. The carbon variation graph reaches a maximum value at this age. The percentage of oxygen has the highest values compared to the other chemical elements, and around the age of 40 showing a minimum. The calcium percentage content variation shows around age of 40 this element a minimal percentage (green line). Around the age of 23, being in the maximum percentage. The third studied chemical element is phosphorus that presents around the age of 23 a maximum value following by a minimum value at around 40 years. Also, this element presents the same variation as the other two ones. The forth chemical element, the carbon, presents a totally different variation compared with the other elements. During time, different variation are recorded but the maximum value is around also the age of 40. Interesting fact is the cyclic maximum and minimum content of carbon. Maximum values are about 24, 34, 43, 54 years old and the minimum inside these periods. The natrium content (gray color) presents a relative uniform variation with not excessive maximum and minimum but as been observed to previous elements, the minimum value is also around the age of 40 while the maximum is around the age of 35...38 years. The last recorded chemical element is magnesium (yellow line). As the same wi th natrium, its variation is relatively constant with the lowest value starting to age 40 towards to 50 years. The maximum is also found around the age of 25.

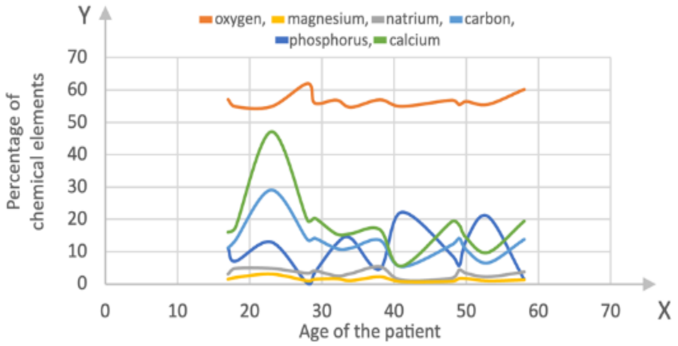

Fig. 8. Variation of chemical composition in dentine

In the case of enamel, the variation of chemical elements according to age is much better defined and distinct (Fig. 9). As in the case of dentin, the **oxygen** content is the highest compared to the other chemical elements. Its minimum is clearly at the age of 40. The second chemical element is **calcium** that presents cyclic variation with clear maximum and minimum values, but not cyclic. Around age of 40 (green line) a very sharp value is recorded, as previous variation. The phosphorus content in enamel (light blue) is relatively constant but with a clearly minimum value around age of 40. For the same, natrium variation from dentine, in enamel, has a relatively uniform variation (gray color).

A very similar, uniform variation, is also observed for magnesium represented with yellow color. The minimum value is also observable for this age. As in the case of the chemical analysis in the dentin structure and in the case of the enamel, the carbon content reaches a maximum value around the age of 40. Moreover, this chemical element has a behavior exactly opposite to the others (dark blue).

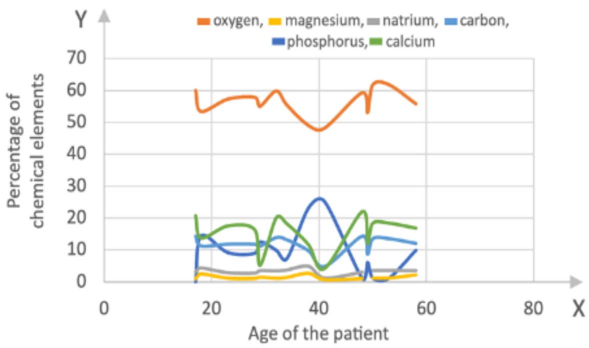

Fig. 9 Variation of chemical composition in enamel

6. Conclusions

This research followed from the very beginning the finding of some interdependencies of the chemical elements that are part of the dentine and enamel of some randomly chosen teeth depending on their age. The existence of this condition among the selected population living around Bucharest is not yet established in this moment. Using SEM microscopy, the chemical compositions of oxygen, carbon, magnesium, silicon, phosphorus and calcium were determined in well-defined, representative areas of dentine and enamel.

The corresponding graphs were drawn for the two areas of the tooth and it was observed that with the exception of carbon, the other elements have minimum values around the age of 40. Instead, carbon shows a maximum value at this value. In this sense, we consider that research can continue by introducing and interpreting other variables, but which would involve a much larger number of tooth samples.

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