CONCENTRATION AND DISTRIBUTION OF TRACE ELEMENTS IN DENTAL ENAMEL USING THE ENERGY-DISPERSIVE X-RAY SPECTROSCOPY TECHNIQUE

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Abstract

The aims of this study were to determine the concentration and identify the distribution of existent trace elements in dental enamel from whole sections of dental organs. In this study the distribution of trace elements was analyzed considering three main areas: the surfaces near the dentin-enamel junction and the external and intermediate surfaces. Seventeen location points were studied in the enamel tissue through scanning electron microscopy. Qualitative and quantitative analysis of chemical elements were performed by spectral dispersive X-ray energy.

The results of the present study indicate the presence of thirteen trace elements (Al, Sn, Sb, I, Si, Yb, Ba, K, Br, Sr, Sc, In, S), their distribution and concentration models are presented here and different anatomical distributions in the crown of the dental organ are shown. In conclusion, with the energy dispersive spectral X-ray analysis, different element distributions and concentrations of trace elements were identified in the thickness of the dental enamel.

Keywords: Dental enamel, Scanning electron microscopy (SEM), Energy dispersive spectral X-ray analysis (EDS), Trace Elements

Introduction

Dental enamel is considered the hardest tissue in the human body, this is due to its high concentration of chemicals elements which consists of

96% inorganic material and 4% organic material of which approximately half is water (Silverstone & Poole, 1968, Gwinett, 1996, Pugach et al., 2010, Soares et al., 2010).

It has been observed that the crystalline structure of the inorganic content of the enamel is hydroxyapatite: [Ca10(PO4)6(OH)2] (Elliott, 1973, LeGeros, 1981), which is also found in bones, calcified cartilage, dentin and dental cement. Several trace elements such as strontium (Sr), magnesium (Mg), lead (Pb), sodium (Na) and fluorine (F) can be incorporated during the formation of enamel or when present in the oral environment, and are absorbed by the enamel prisms and incorporated on to their surface (Weatherell et al., 1968, Robinson et al., 1981, Sakae 1988).The highly calcified enamel structure is likely to be biologically degraded by the corrosion caused by acids, which is a result of the transformation of carbohydrates by bacteria found in the oral environment. Demineralization constitutes the first phase of the generation of caries and it is followed by the dissolution of organic material.

There are analysis reports about the dental enamel inorganic content by chemical analysis (Zheng et al., 1997), laser ablation induction-coupled plasma mass spectrometry (Dolphin et al., 2005) and atomic absorption (Giambro et al., 1995) in which the chemical elements that constitute the dental enamel are detected.

The analysis of dental enamel has been realized using different methods from which various chemical components in the tissue have been observed, these components include: Phosphorus(P), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Lead (Pb), Cobalt (Co), Fluorine(F), Iron (Fe), Aluminum (Al), Selenium (Se) (Deuch et al., 1998, DiekWisch, 1998, Eanes, 1976). The chemical composition of pulverized dental enamel by chemical analysis according to Orban is a 2.3% of water, 1.7% of organic material and a 96% of ashes, Regarding to the ash composition, is such that in a hundred grams of it, it is find: 36.1 Ca, 17.3 P, 3.0 Carbon Oxide, 0.5 Mg, 0.2 Na, 0.3 Potassium (K), 0.016 F, 0.1 Sulfur (S), 0.01 Copper (Cu), 0.016 Zn, 0.003 Silicon (Si) , Beside, there were reported Silver (Ag), Strontium (Sr), Barium (Ba) , Chromium (Cr), Manganese (Mn), Vanadium (V), Aluminum (Al), Lithium (Li) and Selenium (Se) minimum amounts (Zheng et al., 1997, Giambro et al., 1995 . Brudevold et al., 1967, Derise et al., 1974, Trautz, 1967., Carlos et al., 1965, Aprile et al., 1962). In the present investigation the scanning electron microscope JEOL 5900 is coupled to a spectrum dispersive X-ray energy scanner (Oxford Instruments, EDS) which was used in order to visualize and analyze all the chemical elements existent in samples.

Results of previous research shows the contents of some chemical elements in pulverized tooth enamel, but it does not show the distribution and concentration of these trace elements in the areas that constitute the

enamel tissue. There are reports about the dental enamel composition; however, no data is presented on the concentration and distribution of trace elements in the thickness of the dental enamel. Previous studies show data on the mineral composition of the dental enamel, but no records are found regarding their location in different areas and depths of dental enamel thickness. In this study we aimed to establish the presence of trace elements in the enamel thickness of human teeth in a systematic manner, obtaining the quantitative analysis of trace elements in the samples taken from tooth sections; taking into account 17 specific points, including the width and length of the thickness of the adamantine tissue. (Fig. 1)

Materials and methods

Ten healthy premolars, extracted due to orthodontic indications were studied; ethical approval from the Ethics Board of the Faculty of Dentistry, University of San Luis Potosí under the legal framework in Mexico was obtained in order to handle all the dental organs involved in the study. From each organ was obtained a slice along the longitudinal axis, and extracted with an abrasive wear technique (Flexible Diamond Discs, KG Sorensen, Sao Paulo, Brazil), obtaining a flat and polished sample. In order to avoid surface contamination, the samples were washed in sterile deionized water and filtered through a 0.22µm membrane (Millipore) (Loyola Rodriguez et al., 2010). This was done to wash out the residues from the process. Afterwards, in order to develop the qualitative and quantitative analysis, the process of dehydration was carried out: drying (Critical Point Drier, EMCPD030, Leica, USA) and coating the sample with carbon using the DentumVacum II.

The samples were examined through scanning electron microscopy (SEM) in a JEOL 5900 system configured with an EDS detector operating at 20-kV accelerating voltage and 10 mm working distance (Figure 1). The EDS data were compiled for analysis using the Link ISIS system (Oxford Instruments, UK). The images were initially taken with a SEM with a magnification of 200µm (Figure 2A), afterwards the points of study were determined and analyzed with the EDS with a magnification of 20µm (Fig. 2B). This resulted in a histogram, where the horizontal axis displays units of energy [kilo electron volts (keV)] and the vertical axis represent the intensity. (Fig. 2C)

A descriptive analysis was realized on 30 dental organs, to which the arithmetic mean and deviation standard was calculated; the values obtained are represented on box plots (Fig. 3, Table 1).

Results

Different inorganic components were observed; the mineral content shows different anatomical distributions in the crown of the dental organ (Fig. 4).

For Iodine and Ytterbium (0.04%), both showed higher concentrations that were identified along the area near to the enamel-dentin junction and showed an average concentration in the outer surface of the enamel. Note that the lowest concentration was identified in the intermediate part of the enamel thickness (Figure 4A-B). For Al (0.1%) it was noted a greater concentration on the outer part of the enamel thickness; the lowest concentration was located near the enamel-dentin junction. Note the absence in the intermediate part of the enamel thickness (Fig. 4C). In the case of Bromine (0.01%) the distribution of this element was only observed in the inner part of the enamel thickness (Figure 4D). Relative to Si (0.06%) it appeared in different location points, but more specifically in the total tissue thickness of the intercuspideal zone. There was presence of silicon in the intermediate part of the enamel thickness in the cervical orientation on both, the buccal and palatal sides of the crown (Figure 4E). For Tin (0.19%) and K (0.09%) there were different location points showing a homogenous distribution in the enamel thickness (Figure 4F-G). Sr (0.09%) was identified mostly in the inner part of the enamel thickness near the enamel-dentin junction, and markedly on the tip of the cusps (Fig. 4H). Scandium (0.009%) was located in the intercuspideal zone of the enamel thickness in the three points studied: inner, intermediate and outer parts of the enamel thickness. It was also found isolated in the inner point of the enamel thickness in the buccal cusp (Fig. 4I). Presence of Antimony (0.05%) was identified in the intermediate point in both cusps and the inner point of the buccal cusp (Fig. 4J). Barium (0.08%) was located only in the intermediate point of enamel thickness in the middle of both, buccal and palatal sides (Fig. 4K).

Sulfur (0.001%) was located in the intermediate points in the intercuspideal zone and the buccal cusp (Figure 4L). Finally, Indian (0.002%) was shown only in the intermediate point of the enamel thickness in the intercuspideal zone (Figure 4M)

Discussion

In the results of this research, different patterns of distribution and concentration of trace elements in the tooth enamel thickness can be observed, due to the nature of the research design through the utilization of different points of study (Fig. 1); this was only possible due to the EDS technique coupled to the SEM. Trace elements with different values than those cited by Orban were observed (Aprile et al., 1962), demonstrating minor concentrations. The results agree with only 5 of the 17 trace elements

of the previously cited author, probably due to the obtainment of the sample through wear and not by pulverized enamel, or maybe due to the geographical site where the samples were obtained; therefore differences between humans of distinct regions could be observed.

Once the enamel matrix commences to be secreted by the ameloblasts and afterwards mineralized, this matrix which is subsequently degraded by enzymes, specifically by metalloproteinase 20 (Sun et al., 2008), initiating the process of enamel mineralization. At the end of the production of the organic matrix and once the tissue is calcified, the ameloblasts involute by apoptosis. Consequently, once the enamel tissue is formed, the cells involute and the tissue are irreplaceable.

In healthy enamel the Retziusstriae can be observed. These indicate the periods of rest during the mineralization between the layers of enamel, extending from the dentin-enamel junction to the outer surface of enamel tissue. In the present investigation different location points of the elements described above were observed, we assume that during those periods of calcification there was absorption of various ions.

Regarding the presence of metals described in the results, which were found in low concentrations, it can be inferred that when carbonates and sulphates are created, whose nature is anionic which allows the binding of metals, they end up being deposited in the dental enamel.

On the other hand, it has been observed that the permeability of enamel is gradually lost with the passage of time. It is clear that it acts as a semipermeable membrane when the enamel is young; this property allows the slow passage of water and small molecular substances through spaces or pores between the crystals. These pores decrease with age and the crystals acquire more ions and increase in size. These ions can be incorporated to the enamel tissue due to geographical reasons. Natural contamination has been found in seventeen states of the central and southwestern zone of the Mexican Republic, due to the presence of chemicals in groundwater.
This contamination is geological in nature, resulting from the natural interaction of groundwater with some volcanic rocks that are widespread in the Sierra Madre Occidental, which constitutes one of the major aquifers from which water is provided to the people.

In addition, the lack of superficial fluid has forced 75% of the population to extract groundwater that should not be used for human consumption. Some of the metals described in our results (aluminum, barium, strontium, ytterbium and scandium) can be accumulated in the earth and water, something that consequently results in an increase in the concentrations of these elements in humans and subsequently in the dental organs. In conclusion, the chemical elements found may have a food or water source.

In each individual, the presence or absence of one or another trace element in the dental enamel may depend on their diet; as well as on the type of water present in the area the individual inhabits because this is a determining factor for finding specific trace elements. Such is the case of the use of iodized salt, which is commonly used among the population as a daily source of iodine.

In relation to fluorine, a control test with sodium fluoride was performed that demonstrated the capacity of detection of this element by the EDS technique used for the detection of the other element; the results of this investigation did not detect fluorine in the dental enamel samples studied with the previously mentioned technique, compared to the studies performed through chemical analysis (Zheng et al., 1997), laser ablation induction coupled plasma (Dolphin et al., 2005) and atomic absorption (Giambro et al., 1995). In relation to the above, surely no fluorine was detected since the dental organs in the study were considered healthy, and probably the possibilities of ingestion of fluorine by water, toothpastes or foods were reduced or null throughout the development of the dental organ.

Derise et al. (1974) mentions a major concentration of fluorine in teeth with an age of 25 years and above, which suggests that fluorides are deposited in the enamel after the tooth´s eruption and throughout life. The studied samples in our study were obtained from patients whose ages ranged between 10 and 15 years, which supports the above hypothesis.

Conclusion

We suggest future research on ion uptake in tooth enamel and the role they could carry out in the physicochemical properties of it. When the basic composition of the enamel by hydroxyapatite crystals is affected, certainly the strength and function of enamel tissue are altered. Moreover, it is of great interest to monitor the side effects that are traduced in morphological and functional changes when exposed to acids, and the design of biomaterials based on other ions different to the ones that compound the calcium apatite as a preventive or corrective measure against dental caries.

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