Application Note #000396

KeyWords
XPS, Human Tissue, Tooth,
Measurements, Surface Analysis

XPS surface analysis of human tooth samples with EnviroESCA

Human teeth from an adult and a baby were studied using EnviroESCA. The results of surface chemical analysis of the as-received human tissues samples are presented. Neutralization of the insulating biomaterial is accomplished by Environmental Charge Compensation enabling X-ray Photoelectron Spectroscopy (XPS) on tissues as tooth or bone.

Motivation

There are four major tissues making up tooth in humans and other animals. These are tooth enamel, dentin, cementum, and dental pulp. The enamel is the protective layer covering the crown which represents the upper visible part of the tooth.

The most solid and mineralized substance in the body is the tooth enamel which is made of 96% minerals, with hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] as the primary mineral, and 4% of water and organic materials. Its color varies from (grayish) white to yellow.

The hardness of enamel is very important because its main role is to protect the underlying dentin. Another reason for its hardness is that enamel serves as surface for chewing grinding and crushing of food. As protective tooth layer it also must withstand heat and cold.

Enamel is formed on the tooth while the tooth is developing within the gum, before it erupts into the mouth. Once fully formed, it does not contain blood vessels or nerves. Thus, enamel is one of the only body tissues that cannot regenerate itself after being damaged and technically it should last a lifetime.

Over time enamel can break down due to plaque formation or be eroded after extensive exposure of acids and low pH foods. Tooth damage can be repaired to a certain degree by remineralization but further damage beyond that cannot be repaired by the body.

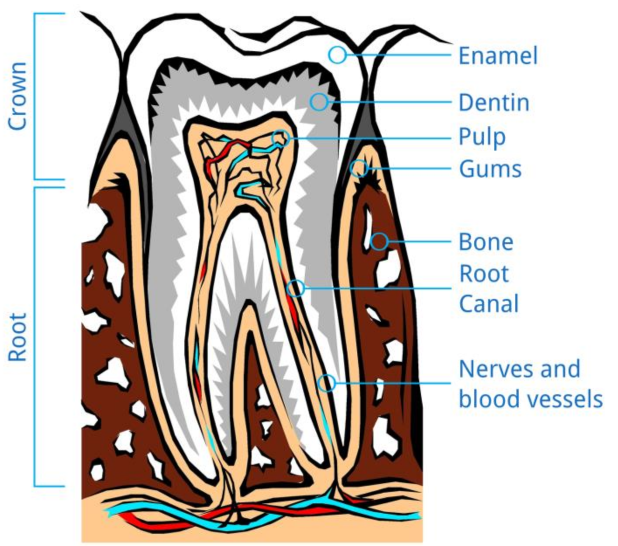


Fig. 1 Cross section illustrating the parts of a human tooth

Method

EnviroESCA utilizes X-ray Photoelectron Spectroscopy (XPS) as its main analytical technique. Hereby an electron beam is generated inside the X-ray source and focused onto an X-ray anode made of aluminum. The deceleration of the electrons on the anode leads to the production of X-rays. This X-ray beam is monochromated and focused onto the sample.

X-ray photons impinging the sample excite electrons in the material which are subsequently emitted with specific kinetic energy determined by their binding energy and the photon energy of the x-rays.

Thereby only electrons from atoms down to a depth of approx. 10nm are able to leave the surface in case of solid samples. These electrons propagate through the lens system of the Electron Analyzer into the hemisphere which acts as a spherical capacitor forcing the electrons onto circular paths with radii depending on their kinetic energy. The path of photoelectrons ends at an electron sensitive detector where the electrons are amplified and measured as intensity in counts per second. Sweeping the voltage of the spherical capacitor while measuring the number of electrons per second on the detector results in a photoelectron spectrum. From these spectra a quantitative analysis of the atomic composition of the sample surface can be done.

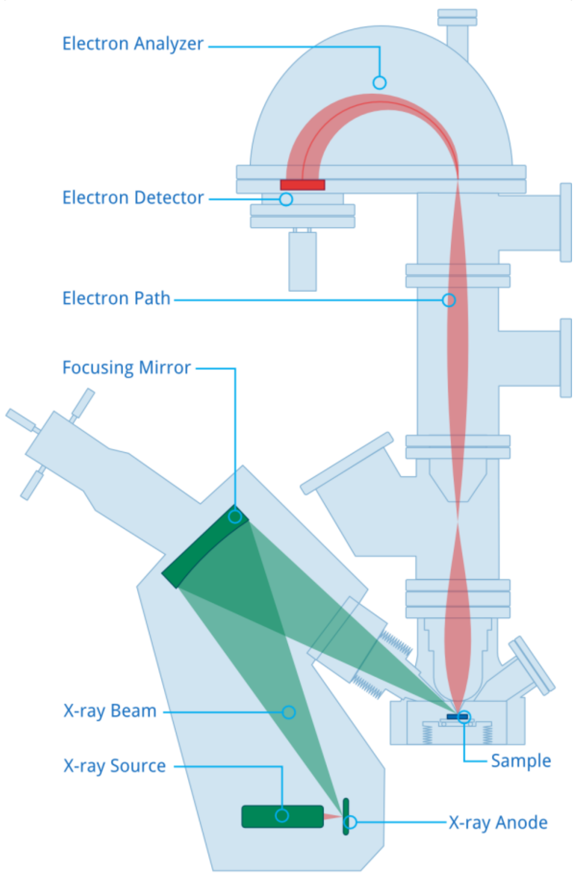


Fig. 2 XPS with EnviroESCA

Experimental Section

EnviroESCA can work in pressures up to several dozens of mbar and therefore does not necessarily require vacuum conditions which overcome the problems of outgassing, drying or special treatment of natural samples.

In classical XPS systems biomaterial tend to charge up quickly under X-ray illumination due to their insulating nature which makes charge compensation inevitable. In classical XPS low energy electron and ion sources are being used in addition to the X-ray source to compensate the surface charge of the surface.

The two human tooth samples, an adult molar and a baby tooth, were placed as received on the sample plate without further fixation or other pretreatments. After introducing the samples and pumping down the samples were investigated with the EnviroESCA at a working pressure of 1 mbar of ambient air to compensate for potential surface charging.

In EnviroESCA an intrinsic charge compensation method which we call Environmental Charge Compensation makes additional electron or ion sources unnecessary. The gas atmosphere that is surrounding the sample delivers all the free charges, when illuminated with the soft X-rays, that is needed to compensate for surface charging (cf. fig. 3 for an illustration).

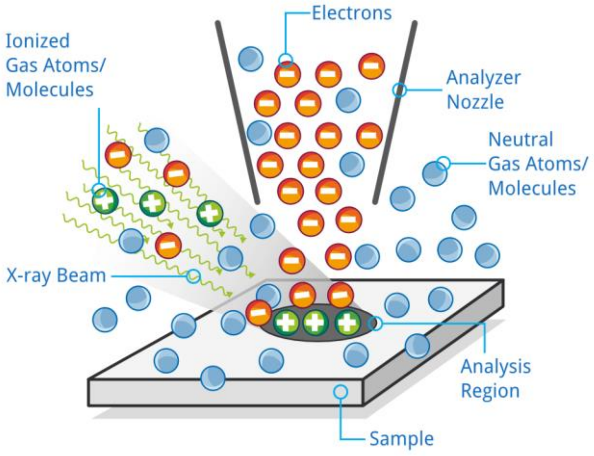


Fig. 3 Environmental Charge Compensation

Results

In the following section we present original unmodified data taken with EnviroESCA. All detail spectra were referenced using the aliphatic component *CC/CH*$_x$ of the C 1s core-level region at 285 eV.

First, a molar tooth from an adult was investigated as-received at 1 mbar using EnviroESCA. Different positions along the tooth were analyzed. The mineral found in human teeth enamel has been described as a calcium-deficient carbonated and fluoridated hydroxyapatite.

The results of the chemical composition analysis are summarized in table 1 together with the data from a hydroxyapatite (HAP, $[Ca_{10}(PO_4)_6(OH)_2]$) reference from literature.[1] The tooth surface is composed of oxygen, carbon, nitrogen, calcium, and phosphor as main elements in accordance with earlier reports.[2,3] Exemplarily the survey spectra of three analyzed regions are shown in Fig. 4.

The chemical composition of the tooth surface differs significantly for the analyzed regions. For example a much higher level of calcium and phosphor are found in the middle of the tooth compared to all the other regions. In this region the cementum is predominant compared to the crown (top region) which is made mainly of tooth enamel.

Additionally to the main elements sulfur and tin were detected in some areas but fluorine was abesent in all regions.

Fluoride treatment has a protective effect because fluoride substitution in the carbonated hydroxyapatite lattice is believed to increase the resistance against acid, thus protecting the tooth enamel against caries.

The detected tin is enriched in the top region (crown) and might be a result of special tooth treatment. *Hercules* and *Craig* reported that teeth treated with stannous fluoride exhibit a tin rich layer at the outer surface.[4]

Other studies concerning the stannous fluoride-hydroxyapatite system suggested that $Sn_3F_3PO_4$ is a major component of enamel surfaces treated with stannous fluoride. Depending on the acidity of the environment further tin based compounds as $Sn_2(OH)PO_4$ or $Sn_3(PO_4)_2$ could be identified.[4]

Table 1. Elemental composition (atom-%) of human tooth samples from an adult and a baby studied with EnviroESCA at 1 mbar working pressure. Also shown are results from a hydroxyapatite (HAP, $Ca_{10}(PO_4)_6OH_2$) reference.[1]

sample	region	O	C	Ca	P	N	S	Sn
adult	**top**	33.9	50.0	2.1	2.5	8.8	1.4	1.3
	middle	45.4	22.3	13.4	10.6	8.0	–	0.3
	root	23.6	54.4	3.8	3.1	13.9	1.0	0.2
	concrement	25.6	51.2	4.8	4.4	14.0	–	–
baby	**top**	18.0	62.6	3.7	4.4	11.3	–	–
HAP[2]		30.9	49.5	6.0	3.9	7.7	0.6	–

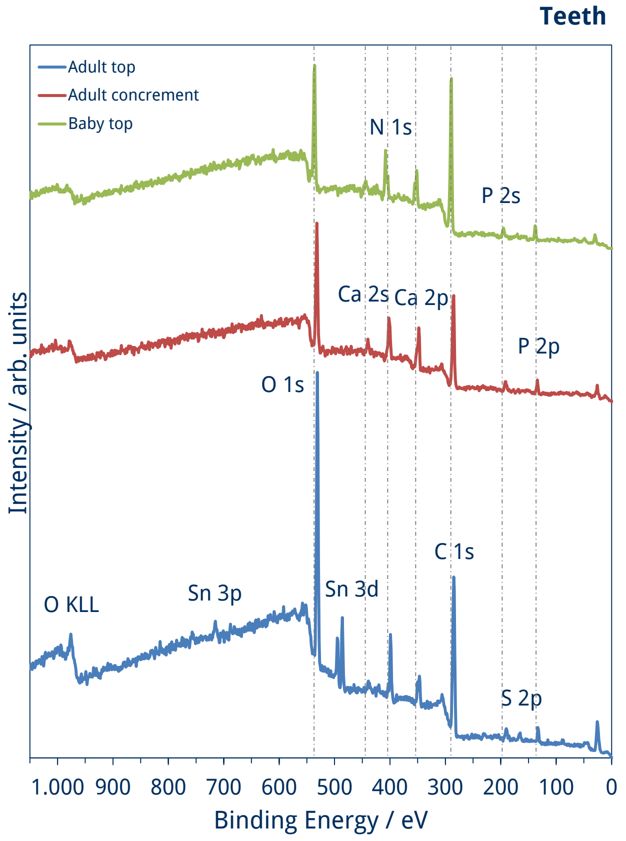
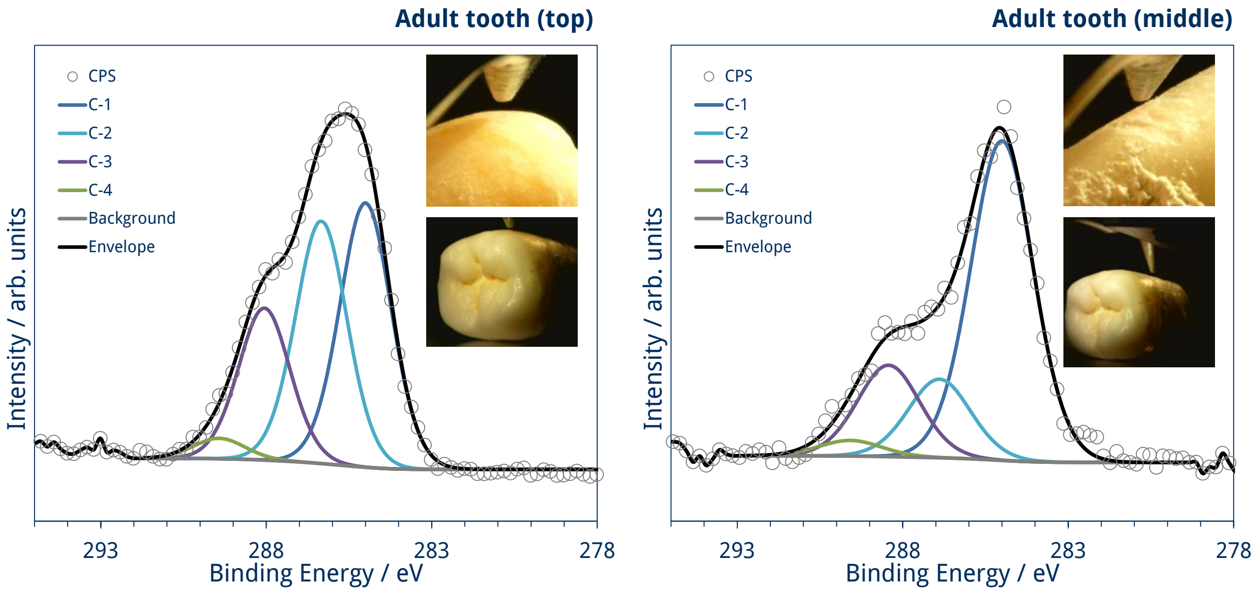


Fig. 4 Exemplary survey spectra of an adult tooth sample at the top (crown, blue), a location in the middle with concrement (red), and a baby tooth sample at the top (crown, green)

Comparable to the observed differences of elemental compositons in the survey spectra the high-resolution detail spectra show significant variations depending on the analyzed surface regions and the sampled tooth.

For example the C 1s core levels, shown in Fig. 5, that were obtained from different areas and samples exibit very diverging peak shapes and peak component area distributions.

The C 1s core-level spectra of the adult tooth are mainly characterized by varying ratios of the C-1 and C-2 peak component. In contrast to that is the C 1s photoemission of the baby tooth dominated by a very intense C-1 component at 285.0 eV that is characteristic of aliphatic carbon atoms *CC/CH$_x$*.



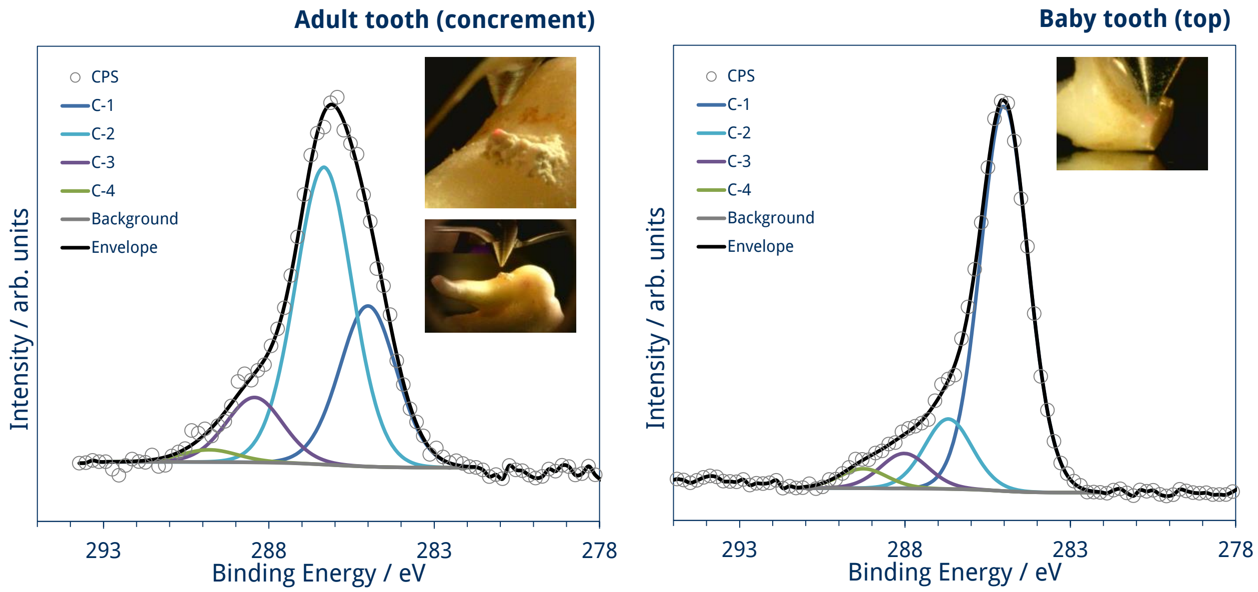


Fig. 5 C 1s core-level spectra of different areas analyzed on an adult molar tooth and a baby tooth sample at 1 mbar

Conclusion

EnviroESCAs ability to work with human tissue samples in near-ambient pressures conditions opens new ways to investigate and characterize human and other biological materials under real world conditions. EnviroESCA's new and easy to use charge compensation method prevents surface charging of otherwise challenging specimens.

The possibility to study human tooth samples without special (pre)treatment or sample preparation was demonstrated with two examples, an adult and a baby tooth. Both teeth showed different chemical compositions depending on their age and the investigated region.

Ethical Remark

The teeth used in this study are voluntary donations by the owners. Thus this study is done according to the common ethical rules fixed by the European Comission.

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