Application Note #000397

KeyWords
XPS, Marine Sample, Mineral, Bone, Measurements, Surface Analysis

EnviroESCA™

XPS surface analysis of cuttlefish bone samples with EnviroESCA

Biological mineral samples from a cuttlefish (sepia) were studied using EnviroESCA. The results of surface chemical analysis of the native and ion implantation treated samples are presented. Neutralization of the insulating biomaterial is accomplished by Environmental Charge Compensation enabling X-ray Photoelectron Spectroscopy (XPS) on tissue samples.

Motivation

Cuttlefish bone, the buoyancy device of cuttlefish, is a natural material mainly composed of calcium carbonate. The internal lamellar matrix of the cuttlefish bone consists of interconnecting parallel sheets with pores of diameter about 200 – 600 micron. As the chemical composition and the highly porous structure of cuttlefish bone resembles those of human bones, the cuttlefish bone is considered to be a prospective material for bone tissue engineering.

Immobilization of extracellular matrix (ECM) proteins and growth factors on the cuttlefish bone surface can help to emulate the natural cell environment, to improve cell adhesion and proliferation, to stimulate specific cellular responses and vascularization and to direct new tissue formation. To obtain a stable protein coating and minimize non-specific protein binding, the surface of the cuttlefish bone was modified using plasma immersion ion implantation (PIII). Plasma treatment with energetic ions creates a high concentration of embedded radicals in the modified layer. The buried radicals (unpaired electrons) diffuse to the cuttlefish bone surface and form covalent bonds with bioactive molecules contacting the surface. PIII treated cuttlefish bone has improved wettability and protein binding capacity. [1,2]

Native cuttlefish bone samples were implanted with nitrogen ions utilizing PIII to create radicals on its surface and in its subsurface.

Fig. 1 Cuttlefish in its natural habitat (top) and the bone (bottom)

Method

EnviroESCA utilizes X-ray Photoelectron Spectroscopy (XPS) as analytical technique. Here an electron beam is generated inside the X-ray source and focused on an aluminum X-ray anode. The deceleration of the electrons on the anode generates X-rays. This X-ray beam is monochromated and focused on the sample.

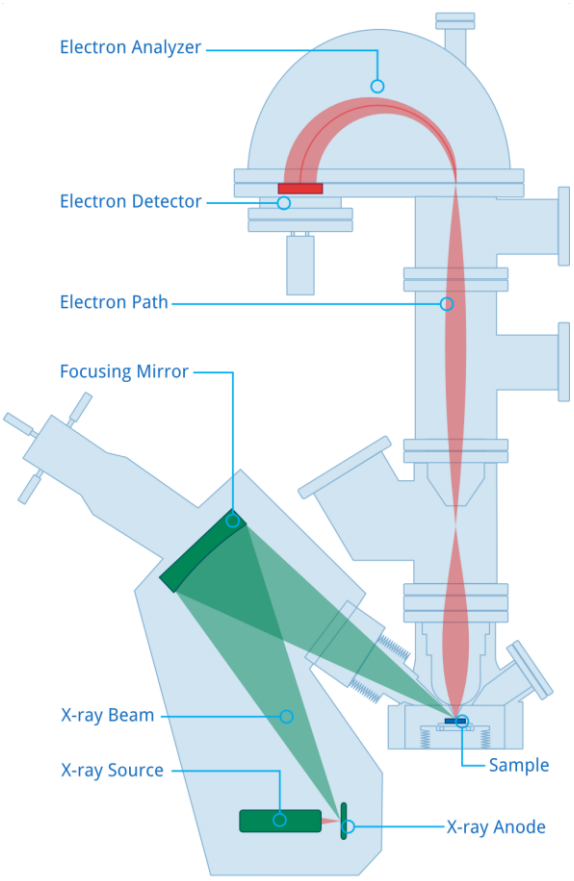

Fig. 2 XPS with EnviroESCA

X-ray photons impinging the sample excite electrons in the material which are subsequently emitted with a specific kinetic energy determined by their binding energy and the photon energy of the X-rays. In case of solid samples only electrons from atoms down to a depth of about 10 nm are able to leave the surface. 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.

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 cuttlefish bone samples, a native and a PIII treated bone, 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 or argon 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. As schematic illustration is shown in Fig. 3.

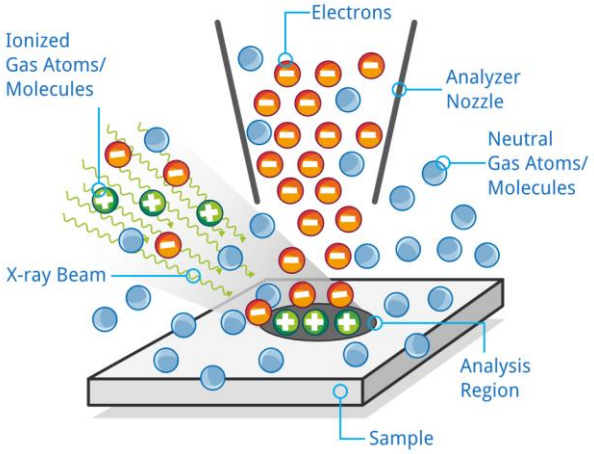

Fig. 3 Environmental Charge Compensation

Results

In the following section we present original data taken with EnviroESCA. All detail spectra were referenced to the aliphatic carbon component C̲C/C̲H$_x$ (Peak 1) located at 285.0 eV.

Calcium, carbon and oxygen were found as main elements in the survey spectra (not shown) of the native cuttlefish bone sample. Additional nitrogen was present in the PIII modified sample reflecting the successful modification of the bone surface due to the nitrogen ion implantation process. The change in surface chemistry can be identified also from the different C 1s core-level spectra of native and PIII modified cuttlefish bone samples, as shown in Fig. 4.

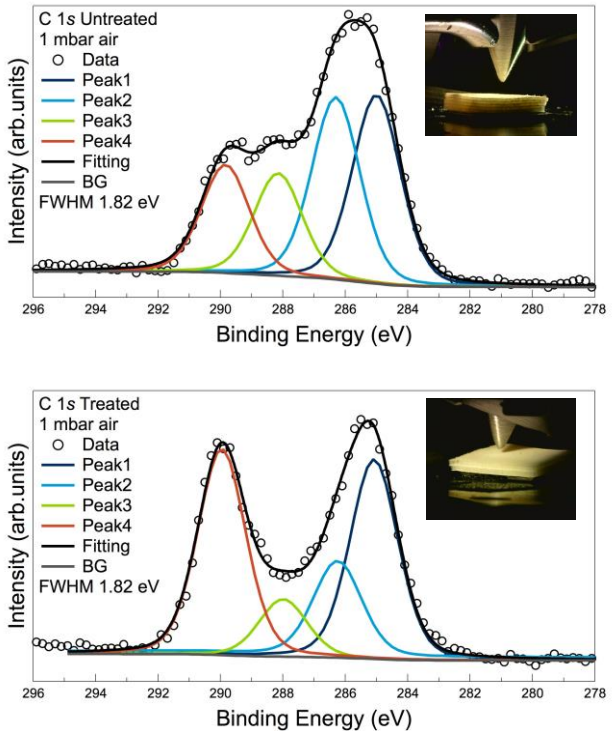

Fig. 4 C 1s detail spectra of native (top) and PIII treated (bottom) cuttlefish bone samples taken at 1 mbar of ambient air. Insets show the respective samples under the analyzer nozzle during XPS measurements.

The process of measuring the ion implanted cuttlefish bone sample in a wet state using an atmosphere of water vapor is illustrated in Fig. 5. The PIII modified sample was placed on a polymer block that was placed in a Petri dish filled with water (Fig. 5a). The analysis compartment was then differentially pumped through the analyzer nozzle.

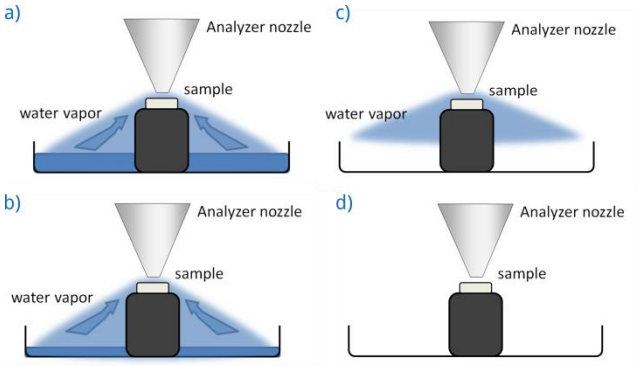

Fig. 5. Illustration of the used measurement set-up inside the EnviroESCA for studying the cuttlefish bone samples under wet conditions in the presence of water vapor and during drying.

Thus, water vapor (continuously evaporating from the Petri dish) passes over the sample surface, ensuring saturation with water adsorbates (Fig. 5b). Since continuous evaporation and removal of gaseous water the amount of liquid water decreases with time. Once the liquid water is completely evaporated (Fig. 5c), the remaining water vapor is also pumped away. And afterwards the adsorbates will successively desorb from the surface (Fig. 5d), which allows the XPS analysis of the *drying* sample surface.

The C 1s core-level spectrum of a wet sample at a water pressure of 3 mbar is shown in Figure 6 (top). The shape is very similar to the spetrum obatined at 1 mbar of ambient air (Fig. 4 bottom) or argon (Fig. 6 bottom) but the individual peak components are resolved much better for a sample in contact with water vapor .

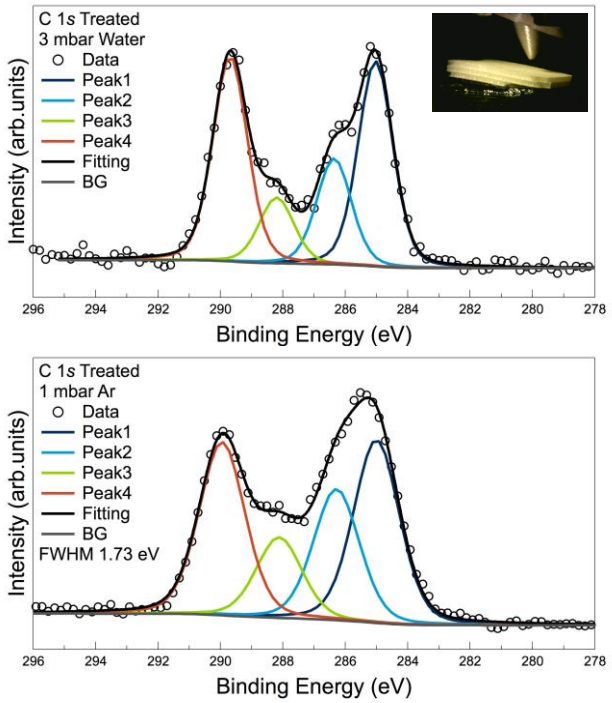

Fig. 6 C 1s detail spectra of PIII treated cuttlefish bone sample taken at 3 mbar of water vapor (top) and at 1 mbar of argon (bottom).

This is a result of a more efficient charge compensation at higher pressures as exemplified by the plot of C 1s peak component FWHM vs. pressure in Figure 7. The FWHM values are in the range of 1.0 to 1.3 eV in a water atmosphere at 3–15 mbar in contrats to FWHMs of about 1.8 eV at 1 mbar of argon or ambient air.

Simultaneously the intensity (peak area) is reduced with increasing working pressure, which means a proper compromise in terms of FWHM and intensity is needed for charge compensation.

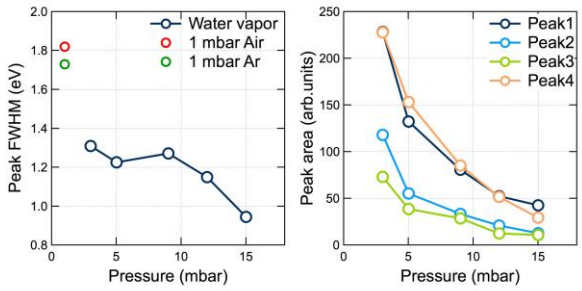

Fig. 7 Plot of FWHM (left) and peak area of the individual C 1s components 1 – 4 (right) as a function of water vapor pressure (3–15 mbar) for an PIII treated cuttlefish bone sample measured under wet conditions.

Conclusion

The unique capability of EnviroESCA to work with highly porous biological samples in near-ambient pressures conditions allows characterization of mineral and bone materials under real world conditions. A new and easy to use charge compensation method within the EnviroESCA prevents surface charging of otherwise challenging specimens.

The possibility to study native and modified biomaterial without special (pre)treatment or preparation was demonstrated on native and modified samples of cuttlefish bone. The cuttlefish bone samples exhibit differences in their chemical compositions depending on the degree of surface modification, here nitrogen ion implantation.

Ethical Remark

No cuttlefish was harmed or killed for this study. This study is done according to the common ethical rules fixed by the European Commission.

[1] A. Kondyurin, M. Bilek, Ion Beam Treatment of Polymers: Application Aspects from Medicine to Space; Elsevier Science, 2014. http://dx.doi.org/10.1016/j.apsusc.2017.04.179

[2] X. Cheng, A. Kondyurin, S. Bao, M. Bilek, L. Ye, *Plasma Immersion Ion Implantation of Polyurethane Shape Memory Polymer: Surface Properties and Protein Immobilization*, Applied Surface Science **2017**, 416, 686-695.

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