XAFS characterization of dogs breast tumors.

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XAFS spectroscopy is a powerful technique for the study of materials lacking periodicity including biomolecules and tissues.[1,2] XAFS, both conventional and micro, has been successfully applied for the study of the bonding of metals in the human nails, which is a modified type of epidermis that consists mainly of keratin.[3,4] Both the soft and the hard (e.g. nails, hair) tissues contain metallic elements in small amounts. Their bonding environment is affected by diseases as it is observed in the case of Fe in neuron tissues.[5,6] Here, we present preliminary XAFS results on the study of the bonding geometry of Fe and Zn in cancerous and healthy breast tissues from dogs. Zn and Fe are significant metallic elements in biological systems due to their catalytic and structural role in proteins.[7,8]

The studied samples (Z78 and Z87) are breast tissues obtained during surgery from dogs suffering from mammary cancer. Non-affected adjacent tissues have been also studied for comparison. The Z78 and Z87 tissues belong to malignant tumors categorized as type I and II (more aggressive), respectively, according to assessment based on morphological criteria.[9] The tissues were conserved in formalin until their measurement. The Fe and Zn K edge XAFS spectra were conducted at the beamline C at room temperature and atmospheric pressure. The spectra were acquired in the fluorescence yield mode using a 7–pixel Si(Li) fluorescence detector cooled at 77 K and positioned on the horizontal plane, at right angle to the beam. The angle of incidence was 45°. The use of an energy dispersive detector allows the discrimination of the Kα fluorescence photons and thus minimizes the background in the XAFS spectra due to the absorption of preceding edges.

Figure 1: Fe K edge (a) pre-edge peak, (b) XANES and (c) Fourier transform of the EXAFS spectra of the studied samples. Representative spectrum from a human nail and spectra of divalent Fe reference compounds are also shown for comparison.
The Fe K edge XAFS spectra of the studied samples are shown in Fig. 1. The XANES spectra are compared with reference compounds of divalent Fe and a spectrum of a human nail in Fig. 1(b). From the position of the absorption edge it is deduced that Fe present in the tissues is mainly trivalent. However, there are not significant differences between the XANES spectra of cancerous and healthy tissues. Compared to the spectrum of the human nail which contains higher amount of S, the spectra of the tissues are characterized by lower intensity in the energy region about 10eV above the absorption edge. The characteristic pre-edge peak that is shown in Fig. 1(a) can be fitted using two or three Lorentzian functions at 7.1114, 7.1135 and 7.1169 keV. The 1\textsuperscript{st} and the 2\textsuperscript{nd} correspond to octahedrally coordinated Fe\textsuperscript{III} while the third is characteristic of Fe-Fe interactions of adjacent octahedra. The valence of Fe is also verified from the Fourier transforms of the EXAFS spectra shown in Fig. 1(c), where smaller distance of Fe with its 1\textsuperscript{st} neighbor is observed compared to the Fe\textsuperscript{II} reference compound. Finally, significant differences are observed in the Fourier transform of the healthy sample from the donor suffering from aggressive mammary cancer. The Zn K edge XANES spectra of the studied samples are shown in Fig. 2(a) along with two representative spectra of human nails. The spectra of reference compounds where Zn is bonded with O, S and N are shown in Fig. 2(b). Slight variations are observed in the spectra of the different tissue samples. The spectra are very similar to the spectrum of “nail1” where Zn in tetrahedrally coordinated with almost 4 N atoms contrary to the “nail2” sample where Zn is tetrahedrally coordinated with 3 N and 1 S atom.[10]

In conclusion, Fe and Zn K edge XAFS spectra of breast tissues have been successfully recorded. Fe is found trivalent and octahedrally coordinated while Zn is found tetrahedrally coordinated mainly with N atoms. Fitting and simulation of the XAFS spectra is in progress in order to identify the amino acids, which are bonded to Fe and Zn and in order to detect disease-induced alterations in their bonding environment.

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References