Experiment SC1461 report
Characterization of normal and pathological bone mineral specimen by XANES

In this experiment, we focused on the elemental composition and structure of the mineral part of "normal" and pathological (Osteogenesis Imperfecta: OI) bone specimen using micro-spectroscopic techniques (XANES and X-ray fluorescence).
On the ID21 beamline, we worked at the Ca edge (4.1 keV) and P edge (2.16 keV) for specimen analyses by X-ray fluorescence and XANES micro-spectroscopies. We obtained:

- XANES spectra of Ca and P in "normal" and pathological bone specimen to examine if Ca and/or P environment varies depending on the patient health.
- X-ray fluorescence spectra of the various bone specimen to identify and semi-quantify major elements (Ca and P) and some minor elements such as Na, Mg, Fe, F.
- Elemental mapping within and around an osteon with a spatial resolution of 1 µm

Materials and method

The bone specimen have been prepared at the Laboratoire de Recherches Orthopédiques UMR CNRS 7052, Faculté de Médecine Lariboisière, Paris, France. They have been fixed, dehydrated and embedded in PMMA resin. The block have been cut into slices (<1 mm of thickness) using high speed microtome saw. Then, bone cross sections were hand polished using first 2400 sandpaper and then alumina slurry (0.5 µm particle size). Further polishing have been done at ESRF with diamond powder (0.25 µm particle size) before analyses. Due to OI bone fragility, the reduction of bone cross sections thickness is limited (thickness > 100 µm) which compromises the analysis in transmission mode and thus affects the quality of the signal collected in the fluorescence mode.
We analyzed 12 specimens from 2 to 17 years old young patients 7 specimens are from young patients without any bone constitutive pathology ("normal") and 5 specimens are from patients suffering from Osteogenesis Imperfecta (1 moderate and 4 severe cases).
We especially focused our investigations in regions of specimen around osteons which are areas of active bone remodelling.

Results

We present in this report the first results obtained for 2 "normal" specimens (20_3 and 33_2) and 2 OI specimens (gal_4 and bal_2) of the same age (6 and 10 years old, respectively). Figure 1 showed the X ray fluorescence spectra of "normal" and pathological specimens in the range of X ray emission lines of some minor elements.

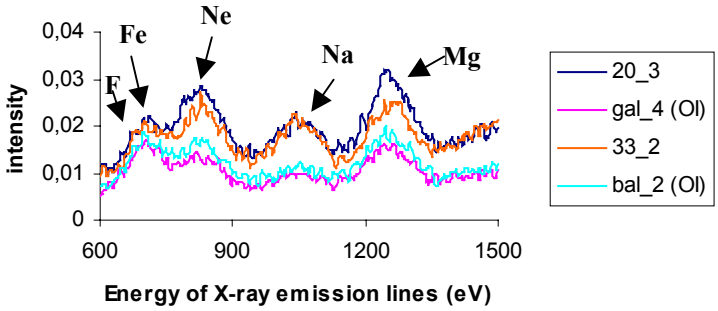


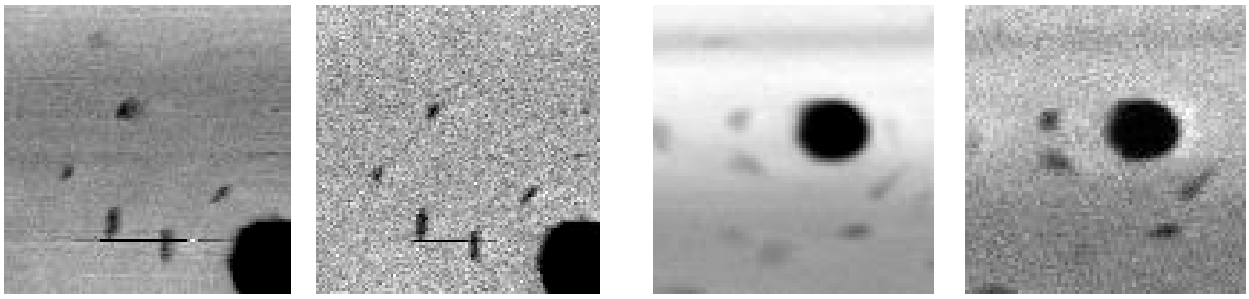
Figure 1 : X-ray micro-fluorescence spectra (600-1500 eV), obtained at the P edge, for OI (gal_4 and bal_2) and "normal" (20_3 et 33_2) specimens.

Systematically, we notice significant difference in peaks intensity between "normal" and OI specimens. In table 1, we reported an evaluation of some minor elements proportion calculated from intensities ratio normalized for each spectrum to the phosphorus peak intensity at 2013 eV. Higher levels of F, Fe and Mg are found in OI specimens whereas the level of Na is lower.

Table 1 : Semi-quantitative evaluation of the proportion of some minor elements (F, Fe, Na and Mg) found in "normal" and OI specimens of the same age from X ray micro-fluorescence spectra at P edge (figure 1).

Biological specimens	Age (years)	F/P 10^{-3}	Fe/P 10^{-3}	Na/P 10^{-3}	Mg/P 10^{-3}
33_2	6.2	3.35	7.08	3.91	4.32
20_3	10.3	2.84	6.37	3.44	4.65
bal_2 (moderate OI)	6.6	4.01	8.77	2.89	4.26
gal_4 (severe OI)	10	4.69	11.10	3.12	5.22

Ca and P elemental mappings around osteon are presented in figure 2. It seemed that there is homogeneous distribution of Ca and Pboth in “normal” and pathological specimens of children of 10 years old (20_3 and gal_4 specimens).



a) **b)** **c)** **d)**

Figure 2 : Ca (a and c) and P (b and d) elemental mapping in "normal" (a and b) and OI (c and d) specimens from X ray fluorescence micro-spectroscopy.

Figure 3 shows XANES spectra (after normalization of the jump between 4 and 4.15 keV) of the same "normal" and OI specimens. Several similar and different features can be noticed by examining these spectra (splitting and intensity of the white line, presence of 2 pre-peak).

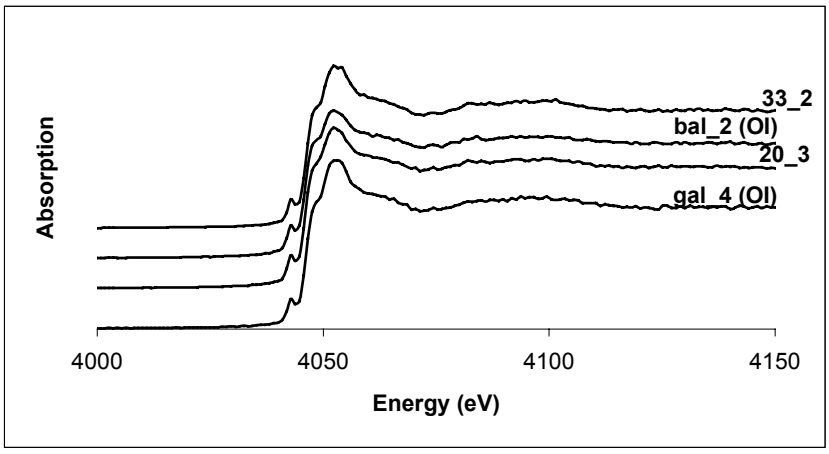


Figure 3 : XANES spectra (4000-4150 eV) obtained at Ca edge for "normal" and OI specimens.

Further experiment data treatments and investigations on such specimens :

XANES spectra at higher energies (4060-4100 eV) will also be examined after mathematical treatment of the spectra (second derivative).

The treatment of the X ray fluorescence and XANES data of all the specimens analyzed during this experiment is in progress.

Complementary characterization have to be done on the specimens especially by FTIR micro-spectroscopy.