Standard Project

Experimental Report

Proposal title: Selenium Speciation in Articular Cartilage Maturation and Development Process		**Proposal number: 30-02-1093**
Beamline: FAME – BM30B	**Date(s) of experiment:** from: 02/07/2014 to: 07/07/2014	**Date of report:** 25/08/2015
Shifts: 15	**Local contact(s): Olivier Proux**	*Date of submission: September 2014*

Objective & expected results (less than 10 lines):

The benefit of Se to reverse cartilage degeneration is still debated. Se incorporation and the effects of different chemical form of Se on cartilage maturation are unknown. The proposed experiment forms part of an ongoing systematic study, including the use of AFM-Tip enhanced Raman spectroscopy, biological studies and different microscopy techniques , of the incorporation of selenium into cartilage and its constituents, chondrocytes, aggrecans and collagen. The aim of this proposal is to better characterize Se chemical form and particularly oxidation state by High Energy Resolution Fluorescence Detected X-ray Absorption Spectroscopy (HERFD-XAS) and by FT-IR microscopy (FTIRM) the organic matrix of cartilage during maturation and whether different Se exposures would enhance cartilage repair. Here, **our objective is to study the system cartilage-Se focusing on the effect of the organic matrix of cartilage during maturation when Se is provided by various means and to provide detailed information on Se-oxidation state within the cartilage matrix.**

Results and the conclusions of the study:

Immature articular cartilage explants were prepared from the metacarpophalangeal joint of young bovine steers. We have interest in kashin-beck disease (KBD) that primarily affects cartilage growth and repair in children and adolescents, therefore, bovine immature articular cartilage provides an ideal *in vitro* model to test our hypotheses. 6-mm osteochondrondral cores were excised from joints under sterile conditions and culture in defined serum-free culture conditions. Members of our group have published data to show that the cut edges of these cartilage explants undergo active repair processes, the central core of the explants act as an internal control and replicate normal homeostatic growth mechanisms. Thin samples of cartilage were cryo-embbedded and cryo-milled using a dismembrator in order to obtain a highly thin homogeneous powder. Two sets of samples are prepared: cryo-powder and freeze-dried powder (using a vacuum pump) samples. Bulk powder pellets were then made. After a first ICPMS analysis, the concentration range of the different samples was established from 150 ppb to 2 ppm. Using the FAME beamline in cryo-condition (used of cryostat) with analyser crystals (four Ge-422 crystals) setup, the time needed to obtain the Se-spectra of the different samples was long according to this very low concentration (between 14 to 32 hours per sample). Four samples was analysed during this beamtime: ITS (ITS, insulin transferrin selenium) control and FT-treated ITS samples (about 300 ppb for Se-concentration)., "+Se" control and "+Se" FT-treated samples with selenium supplementation in the culture medium (ITS and sodium selenite) (about 2 ppm in Se-concentration). FT-treated means growth factors treatment that induces accelerated articular cartilage maturation as already published by Khan IM et al. [1]. Due to the long acquisition time for these four samples, we didn't have time to analyse the samples depleted in selenium, and freshly collected immature and mature articular cartilage tissues. We thus concentrated our analysis on having as much as possible good statisitic spectrum for Se-supplemented and control samples.

The main expected result of this experiment is the determination of the selenium composition of the native cartilage and during the maturation of cartilage. The hypothesis that KBD would be linked to a Se-deficiency can be already tested with our approach comparing cartilage maturation in presence or absence of selenium and compared to growth factor treatment. More general output of this experiment will be the importance of selenium in cartilage regeneration for osteoarthritis patients. This study required various techniques to best understand the Se role in cartilage maturation and this is linked to fundamental health research and to environmental problems linked to selenium deficiency. Understanding the behaviour of the different sources of Se is necessary to estimate and predict possible consequences to selenium deficiency, in Europe or China and to propose supplementation action for the concerned populations.

Preliminary results obtained due to XANES spectra analysis on BM30B – FAME beamline:

The goal of this experiment was to identify the selenium species present within articular cartilage explants cultured with different treatments in order to confirm or infirm our hypothesis "Selenocysteine should be a predominant Se-specie in articular cartilage because it is one of the main component of the selenoproteins". Using the new spectroscopic caracteristics of FAME beamline, high photon flux and analyser crystals detection systems, we measured high energy resolved fluorescence detected (HERFD) x-ray absorption near edge spectroscopy (XANES) spectra, from samples highly diluted (< 2 ppm) and reference compounds. The HERFD spectra obtained for references(Figure 1) were successfully compared with the ones already measured by Geraldine Sarret *et al.* [2]. Using peak fit and linear combination of our different references measured during beamtime, it appears that selenocystine is a major selenium specie present within the articular cartilage tissue as it is possible to see in the figure 2. This surprising result seems to be intrinsic to the articular cartilage composition. Even if the diselenide bond appears to be relatively more stable than disulfide bond, a diselenite bond required a large energy to be created. Furthermore, this bond is rare in the biological system. Its role in the articular cartilage tissue is not understood. Biological investigations have to be made to explain the exact role of the selenium in articular cartilage metabolism and maturation.

After a discussion with Olivier Proux and Jean-Louis Hazemann, we have pointed the interest to poursue this study. Our objective will be to study the system cartilage-Se focusing on the effect of the organic matrix of cartilage during maturation when Se is provided by various treatments but also in native conditions (immature and mature). This is also to push further the detection limit of the crystal setup, to obtain a good signal and spectra for highly diluted samples with a Se-concentration below the 300 ppb.

Justification and comments about the use of beam time:
The 15 shifts have been used and 4 samples and 9 references compounds have been analyzed. Thanks to the stability and the high beam flux provided by ESRF synchrotron, quite beautiful and precise spectra have been realized. The data was good and without particular problems. However this set of measurement has to be complete with measurement on native conditions sample.

Publication(s): **Writing in progress about Se detection at ppb concentration - complementary analysis in progress to understand the biological meaning of this result. This study have to be related to other experiment such as immunohistochemistry, qPCR, FTIR mapping and DUV-pSHG analysis in order to obtain a large overview of the Se role in the articular cartilage metabolism.**

Reference:

[1] Khan, I.M., Francis, L., Theobald, P.S., Perni, S., Young, R.D., Prokopovich, P. et al. 2013. "In vitro growth factor-induced bio engineering of mature articular cartilage". *Biomaterials*. 34(5):1478-87 1478-1487.

[2] Geraldine Sarret *et al. "Chemical forms of selenium in the metal-resistant becterium Ralstonia metallidurans CH34 exposed to selenite and selenate" 2005 – HAL-00022629*

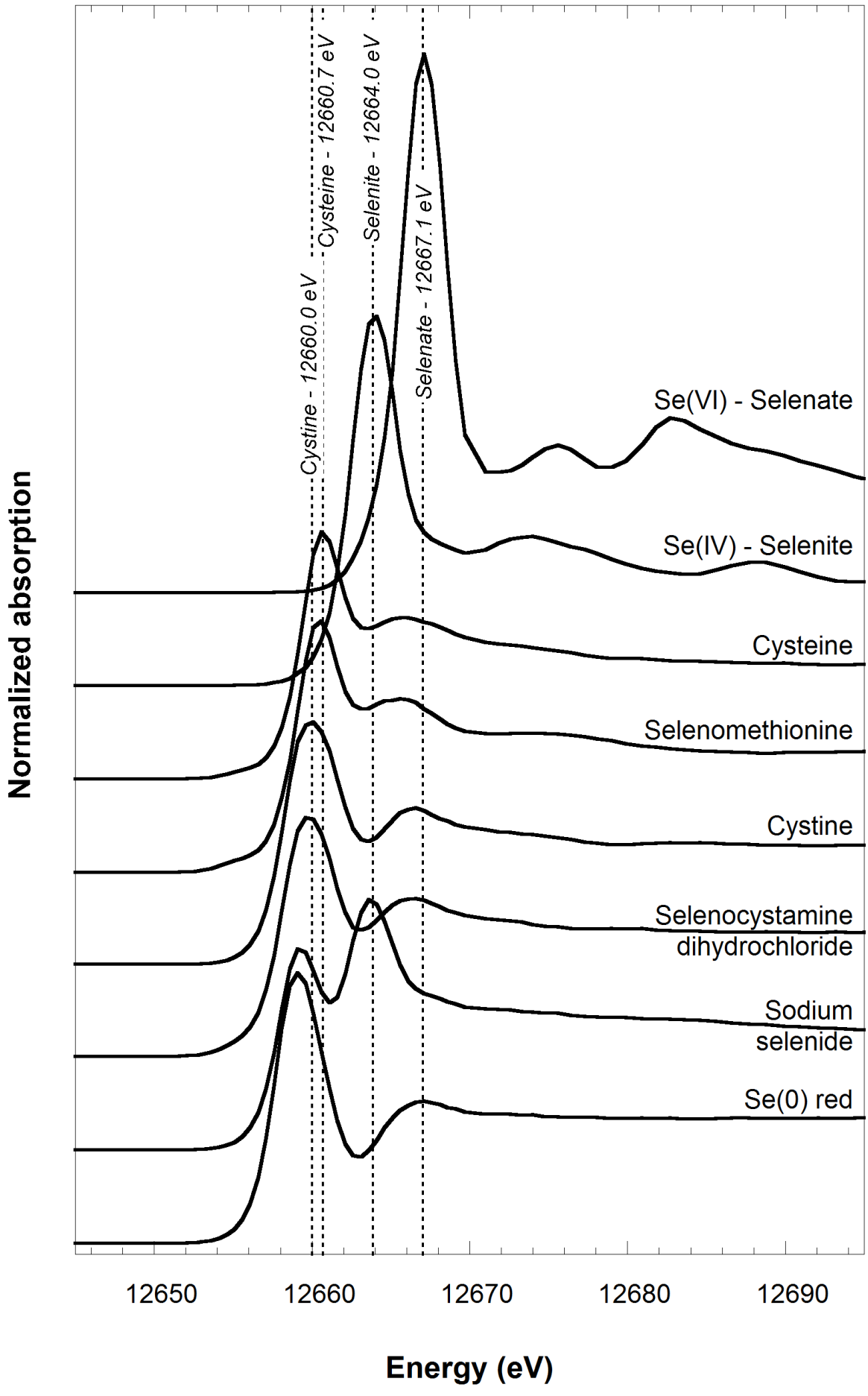

Figure 1: XANES spectra for some reference selenium compounds (in solid state unless specified).

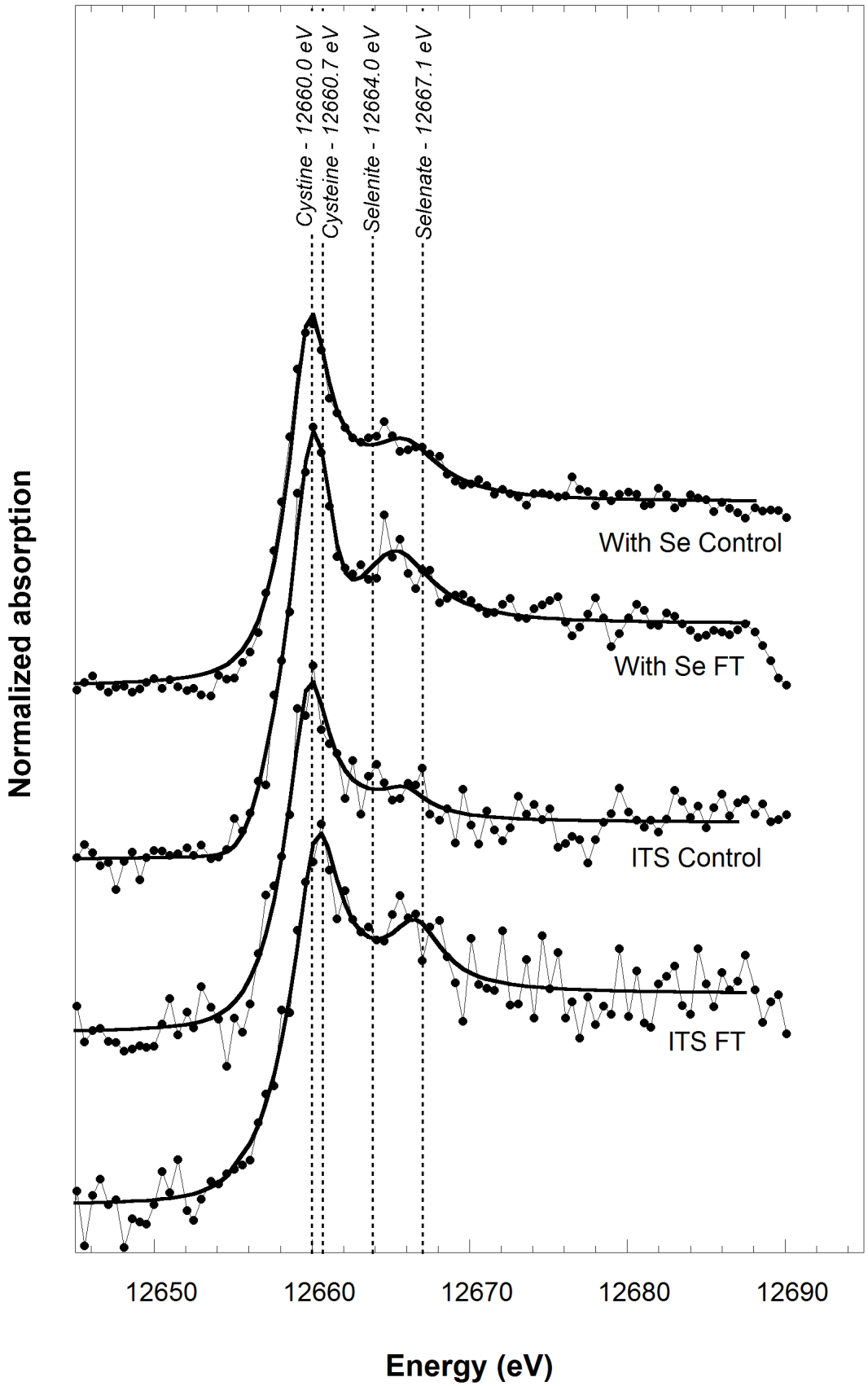

Figure 2: XANES spectra of the different articular cartilage AC samples in solid state. From the bottom to the top: ITS control and ITS FT-treated AC explants (explants treated with ITS – Insulin selenium transferrin) with a Se-concentration about 300 ppb and with Se Control and with Se FT-treated AC (explants treated with a culture medium containing ITS and sodium selenite) with a Se-concentration about 2 ppm. FT means growth factor treatments in order to induce a maturation of the tissue.