ESRF	**Experiment title:** Accessing orientation and residual strains in biogenic apatite biocomposites in teeth by means of X-ray absorption spectroscopy	**Experiment number**: MA3262
Beamline: ID21	**Date of experiment**: 31/08/2016-05/09/2016	**Date of report**: 20/02/2017
Shifts: 15	**Local contact(s)**: **Bernhard Hesse**	

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Background:

Human tooth dentine is a composite biomaterial with impressive, damage-resistant mechanical properties, strongly affected by the mineral (dahllite) component. Recent studies found, that the carbonated hydroxyapatite (cHAP) crystals are in fact compressed by collagen due to a very strong bond. Once the link is broken (e.g. through heating) the co-aligned cHAP nanoparticles relax, resulting in a change of the c-axis parameter [1]. At ID21 of the ESRF, the Ca-K-edge μXANES sensitivity to Ca-containing crystal orientations in bone-like tissues such as dentine, has been shown by analyzing the 1s to 4p transitions [2]. Briefly, the intensity of the XAS profile white line varies as a function of the angle between the beamline polarization vector and the mean orientation of the cHAP crystals. In this Experiment μXANES at the Ca K-edge was performed to assess interaction between the polarized beam and different hydroxyl-apatite crystal texture/orientations and to observe how changes to the c-lattice parameter (altered due to thermal annealing or X-ray induced radiation damage) are reflected in XAS spectra.

Experiments

The beamline was set up using: m_o:Ni, 2 Undulators, mono: Si(111), E=4.1keV, beamsize: 0.7 μm x 0.35μm. Ca-K-edge μXANES spectra (400 energy steps from 4.030 to 4.129 keV at 100ms acquisition time per step) and XRF-maps were collected from cross-sections of ground and polished human roots. A total of five dentin samples were investigated in 15 shifts. To explore the effects of different polarization angles of the incoming X-ray beam (P) with respect to the crystal c-axis vector, a first series of experiments included manual rotation of the sample by 0°, 45° and 90° and re-scanned the same regions. Due to the excessive efforts involved in this approach, we opted to use the tubules as landmarks for the collagen orientation reference. We thus mapped multiple regions where beam polarization vector and collagen fiber orientation were either perpendicular or orthogonal. In each of the samples at least two regions were mapped.

Additionally, we explored the time resolved damage to the collagen by high resolution raster scanning. Some regions were mapped up to 80 times (5x25μm areas, 0.7x0.35μm stepsize, 80ms exposures, 40 repetaed scans per Energy: E1=4.0533, E2=4.0553). In each region at least two XANES-spectra were also acquired. Subsequently larger, encasing maps sized 50μm x 50μm were collected (80 ms exposure time, 0.7x0.35μm stepsize) at three different energies: E1=4.0533, E2=4.0553 and E3=4.2 keV. The XRF signal of the large map reveals higher intensities in the regions previously raster scanned (Fig. 1).

To further observe dose-dependent changes in the collagen-crystal crosslinking 20 XANES spectra were collected at the same position for each sample, with the dose proportional to 800 s of acquisition times. The spectra show a systematic decrease of the white line intensity. Later SEM imaging revealed that the sample surface was partially altered after collection of the XANES spectra (Fig. 1,i). Thus, at the current state of our analysis we are not sure whether XAS effects are due to beam induced changes in the collagen-crystal cross linking with a resulting change in c-axis parameter, or due to sample surface alterations.

In addition, two samples were annealed for 2h at 250°C to damage collagen-crystal bonding[1]. Concomitantly, these samples were investigated in the same manner as described above.

Concluding preliminary observations:

- We could verify earlier assumptions and show that polarization XAS mapping is sensitive enough to reveal dentin crystal orientation changes (paper in preparation[3])
- The collection of XRF maps at the energies mentioned above show a trend of increased intensity (Fig.1 c,d,e) suggesting X-ray beam induced changes to the collagen-crystal bond result in changes to the c-axis parameter (this trend of changes in c-axis parameter we are now investigating by computer simulations (FDMNES), collaboration with C. Sahle, ID20)
- For very long exposures to the energies around 4 keV, the signal in the white line region of the spectra decreases. We suspect that this might be due to alterations of the sample surface through exposure to the X-ray beam or due to beam induced changes of the crystals (for example recrystalization). We will further investigate these effects by using other techniques e.g. by µXRD

Overall the experiment was very successful and confirmed very recent data on the mechanical impact of collagen-crystal binding [1]. In addition we show for the first time how XRF mapping and µXANES spectroscopy at the Ca K-edge can be used to dynamically probe the c-axis parameter changes in biogenic appetites. A publication on our data is in envisaged.

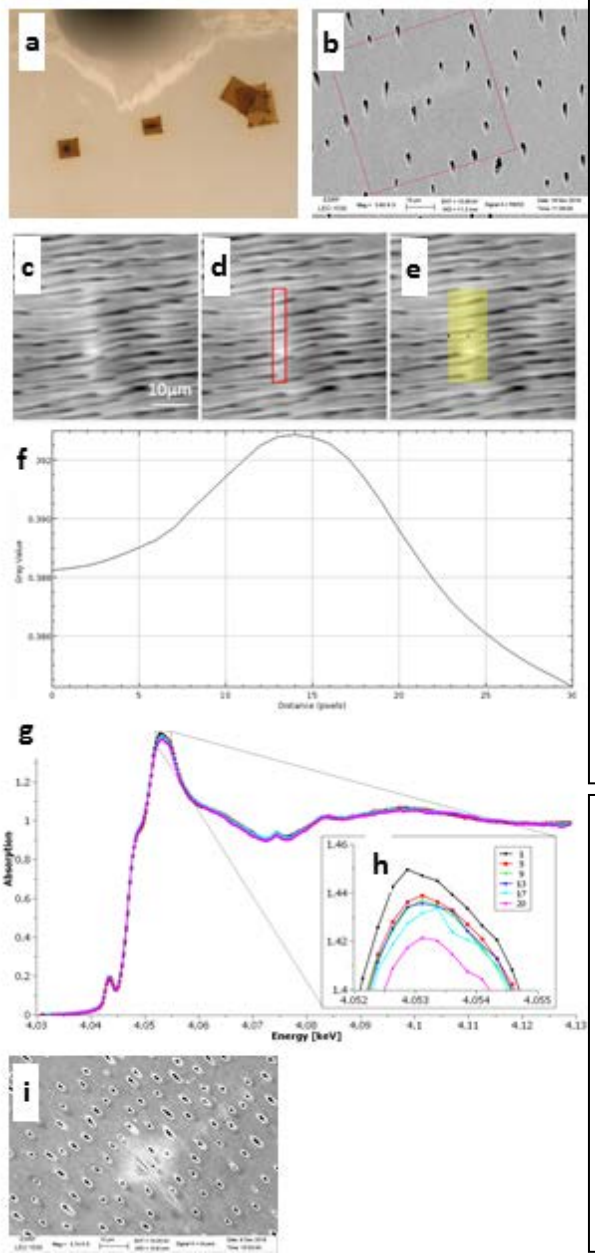

Figure 1. a: Optical images of one tooth section after XRF mapping. After XRF mapping, the mapped regions appear brown.
b: SEM image showing one region after XRF mapping. The region that was 80 times XRF mapped shows different grey-values, however the sample surface geometry appears unchanged.
c-f: XRF intensity changes for long exposure time. c) A large Ca XRF map measured after a series of 80 small sub-maps (marked red in (d)), at E=4055eV. The footprint of the x-ray beam can clearly be seen as a brighter stripe (d). Across the yellow square (e), a line profile across the footprint shows elevated intensity for the higher exposed region (f).
g, h: The development of XANES Spectra (400 points each) measured at the very same sub-micron spot as function of scan-number shows a decrease in the intensity at the white line (h).
i: SEM imaging of the region of which the 20 XANES spectra were collected from reveals alterations of the sample surface.

References:

[1] Forien, J.-B., Zizak, I., Fleck, C., Petersen, A., Fratzl, P., Zolotoyabko, E., et al. (2016). Water-Mediated Collagen and Mineral Nanoparticle Interactions Guide Functional Deformation of Human Tooth Dentin. *Chemistry of Materials, 28*(10), 3416-3427. doi:10.1021/acs.chemmater.6b00811

[2] Hesse B, Salome M, Castillo-Michel H, Cotte M, Fayard B, Sahle CJ, et al. Full-Field Calcium K-Edge X-ray Absorption Near-Edge Structure Spectroscopy on Cortical Bone at the Micron-Scale: Polarization Effects Reveal Mineral Orientation. Analytical Chemistry. 2016;88(7):3826-35.

[3] Hesse B., Stier D., Cotte M., Forien J.-B., Zaslansky P. On the application of polarization sensitivity fluorescence mapping to crystal orientation mapping in human teeth