ESRF	**Experiment title:** The nanostructure of the enthesis and the impact of mechanical unloading on its hierarchical structure, studied by scanning SAXS/WAXS/XRF	**Experiment number**: LS 3153
Beamline: ID13	**Date of experiment**: from: 29.11.2022 to: 04.12.2022	**Date of report**: 25.02.23
Shifts: 9	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Tilman Grünewald (Institut Fresnel, Marseille)*

Sandrine Roffino (Institut des Sciences du Mouvement, Marseille)*

Cécile Genovesio (Faculté de Pharmacie, Marseille)*

Martine Pithioux (Institut des Sciences du Mouvement, Marseille)

Claire Camy (Institut des Sciences du Mouvement, Marseille)

Report:

Summary:

The aim of this experiment was to understand the interplay between prevailing mechanical loading conditions and the nanostructural make up of the bone-tendon interface, the enthesis. The enthesis is a biomechanical interface with immense impact on our day to day life. It creates the load-bearing interface between tendon and bone. It achieves the load transfer by creating gradients of collagen and mineral properties. As this interface is not able to regenerate these gradients once damaged, an accurate understanding of the impact of changing load conditions on the properties of the enthesis is crucial in helping our understanding of the biomechanics of this important structure.

In order to achieve this task, we have investigated the structure of the mouse Achilles tendon enthesis subjected to different loading regimes. This unloading was carried out by controlled hind-limb suspension of the animals. We studied three groups, a mechanically unloaded, an unloading followed by a loading and a control group. We employed μbeam SAXS/WAXS/XRF 2D scanning, and leveraging the fast data acqusition of ID13, providing a statistically relevant dataset by studying 5 samples from each group.

In summary, the experiment unveiled marked changes in the nanostructural makeup of the samples, mostly in the shape and composition of the mineral, but also an enrichement of trace elements like Zn and Sr at the mineralization interface. The collagen fraction proved to be surprisingly difficult to analyse to the directionality of the diffraction signal, calling for a 3D characterization. The first analysis however points to changes in the collagen structure during the unloading and reloading group compared to the control group.

Samples and setup:

The experiment was carried out at the ID13 EH2 microbeam setup. The x-ray energy was set to 17 keV and a set of CRL lenses produced a spot of ~2x2 μm^2 with a flux of ~$8x10^{11}$ ph/s. The Eiger 4M detector was used to detect the scattering signal in two q-range of 0.1 to 45 nm^{-1} for WAXS and in a q-range from 0.1 to 5 nm^{-1} for an improved SAXS q-resolution and dynamics. The XRF signal was detected and the NIST 1577b bovine liver standard was used for intensity calibration. The sample was scanned with the x/y/z scanning setup.

The sample set comprised three groups of samples, the control, unloaded and reloaded sample group. For each of these groups, 10 samples were prepared, of which 5 were measured for each group.

In order improve the q-resolution and to avoid the saturation of the detector on the SAXS region, we chose to measure the same sample at two sample-detector regions, (as indicated above) and rescan the same sample

twice. We carefully established the radiation damage thresholds of collagen and mineral to ensure that the repeated exposures didn't impact the sample.

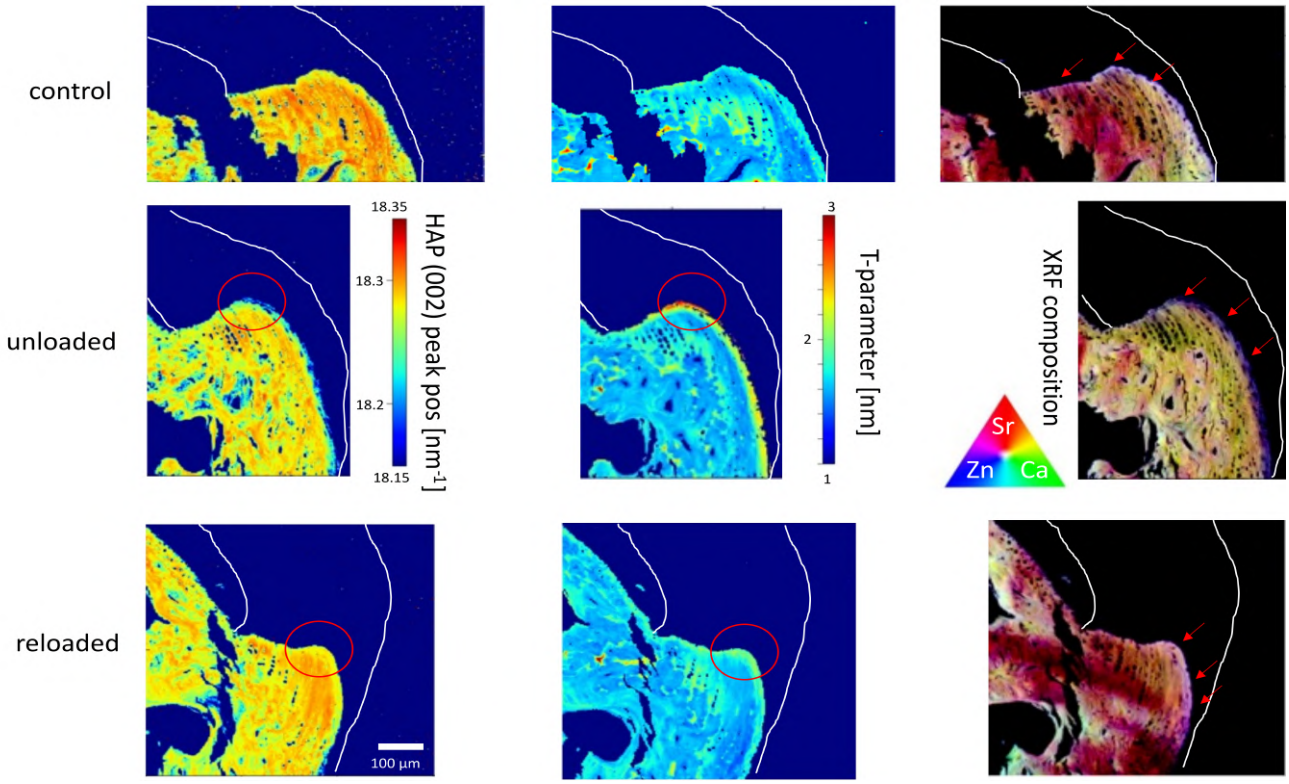

Figure 1 Comparison of mineral properties and elemental composition for the control, the unloaded and the reloaded group, showing the HAP peak position, the mineral particle size and the elemental composition (Sr=red, Zn=blue, Ca=green). Interestingly, the unloaded and the reloaded condition shows a marked shift of the HAP peak position at the interface as well as significantly larger mineral particles (red circles). This goes along with an increase in Zn and Sr content

Principal outcome:

Figure 1 gives an overview over the principal findings of the preliminary analysis of the data. The three groups are compared, the while outlines indicates the full extent of the enthesis and only the mineralized portion is shown here. The HAP peak position at the interface is changing as well as the T-parameter. As this goes along with an enrichment of Zn and Sr, we interpret this at the moment as the signature of increase mineralization turnover. This raises the important question if the mineralization process in the enthesis is governed by the same mechanisms as bone formation and how the mineral fraction is aligning with respect to the collagen fibrils.

The analysis of the collagen fraction and establishing the orientation relation between the two in 2D is currently ongoing as well as the quantitative analysis of the XRF data with the bovine liver standard. An important point here is to assess whether the levels of Zn and SR are sufficiently high to allow a study of the chemical surrounding of these two target elements with X-ray absorption spectroscopy, possible in 3D. A further task is extending the full analysis to the whole dataset to investigate the statistical significance of the observed changes.

Conclusions and further proceedings:

In conclusion, we carried out the experiment successfully and gained important insights into the relation between the mechanical loading regime and the nanostructural properties of the enthesis in 2D. While the data processing is still ongoing, the preliminary analysis shows a marked difference of the interfacial properties and a local heterogeneity which is not fully resolved by the current experimental resolution. We thus wish to expand our experiments to 3D and carry out combined texture and tensor tomography to investigate the mineral properties and the orientational relationship between mineral and collagen further.

Finally, we would like to acknowledge the great support received by our local contact and thank