ESRF	**Experiment title:** Observation of strain-induced micro-cracks in cancellous bone	**Experiment number**: MD 141
Beamline:	**Date of experiment**: from: 16 April 2005 to: 19 April 2005	**Date of report**: 1 Sept 2005
Shifts:	**Local contact(s)**: Eva Pereiro	*Received at ESRF:*

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Report:

Context :The evaluation of bone strength and fragility is receiving increasing attention to explain the mechanism of osteoporosis and bone loss. In addition to decreasing Bone Mineral Density (BMD), bone quality changes during aging and osteoporosis. In particular, the accumulation of micro damage is supposed to act on the mechanical properties of bone [2]. Little is known about the respective contribution of these parameters to bone fragility and to what extent they may serve as markers for the bone maturation and senescence.

Aim of the work : Our goal was to investigate how fatigue-damage micro-cracks propagate within bone. There is only a limited number of studies devoted the evaluation of micro-cracks for which the standard technique is microscopy on stained bone sections. However there are major problems in controlling micro-cracks formation and evidencing them.

Experimental method: We used a new organ culture model to study coordinated bone cellular responses and the resulting tissue response while avoiding complexity of in vivo situation. In this study we used the "Zetos" system, whch is a highly accurate mechanical loading and measurement system combined with a trabecular bone diffusion culture-loading chamber [2]. It provides the ability to culture cancellous bone cores over long periods (until now difficult due to rapid degeneration inside the organ) and to apply specific compressive strains to bone cylinders. The Zetos itself also provide biomechanical parameters.

Strain-induced micro-cracks were studied in cancellous bovine bone envelopes. Cylindrical bovine biopsies (10mm diameter, 5mm height) from sternum were precisely machined, fitted in chambers and fed with culture medium in conditions ensuring uniform double fluorochrome labelling within the whole sample. A compression load was used to induce micro-cracks within trabeculae at the beginning of the culture period (for 3 weeks). We chose a regimen that mimics a jump pattern (4000μS, 1Hz, 300 cycles/d) [4]. The study was performed on basal control (BC) samples, loaded (L), and non loaded (NL) 3-wk cultivated samples.

In order to observe bone micro-architecture and identify micro-cracks, we use synchrotron radiation microtomography (SR μCT) on beamline ID19.

Results : We used the 5µm optic detection system (2048x2048 Frelon camera, pixel size on the detector 4.9µm). To reduce scan time we used the ondulator and energy of 20.5 keV. With these conditions, the available beam height was about 3.7 mm, so that it was necessary to do two scans in each sample.
It was possible to acquire 10 samples from the Loaded group, 10 samples from the Non Loaded group and 5 from the Bascal Control group. After acquisition, each stack was reconstructed, corrected and merged to get the full 3D image.
After pre-processing we get relatively larges 3D images (about 1300*1100*1450 voxels). These images were then analyzed using our own developed software for getting micro-architecture parameters. The following parameters were calculated : BV/TV, BS/BV, Tb.Th, Tb.Sp, Tb.n, Euler Number and SMI index. The statistical analysis did not show significant differences between the groups. Figure 1 illustrates the 3D display of a typical bone volume, and Figure 2 the Box plot of one parameter (trabecular thicknes (Tb.Th)) on the two groups.

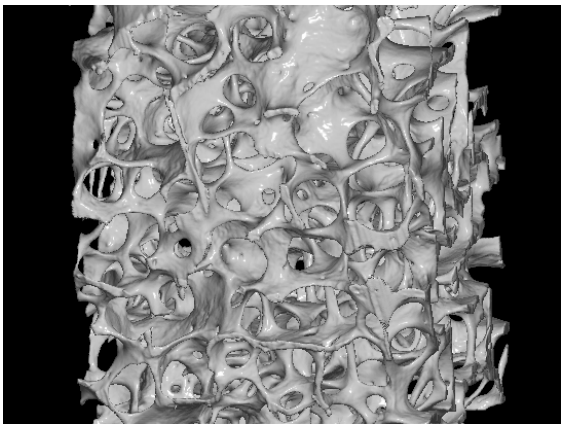

Figure 1 : 3D display of a sub volume in one typical trabecular bovine sample

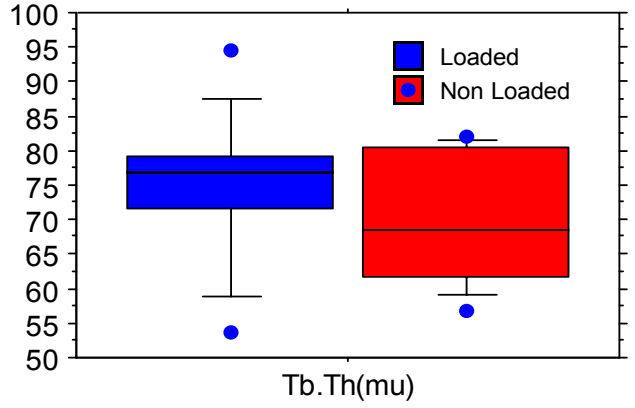

Figure 2 : Box plot of trabecular thickness parameter (Tb.Th) on the two groups.

The micro-cracks were difficult to observe at the 5µm scale. This, during experiment MD141, we also changed the detection setup to made a preliminary test at higher spatial resolution (1.4 µm). We used a subset of samples of smaller size (3mmx3mmx10mm) in order the scan the center core. The first images appeared to be very noisy due to the presence of bone remains during cutting. Cleaning the samples with ultrasound improved the image but did not eliminate all artefacts. Nevetheless, Figure 3 present an example of image were micro-craks could be observed . Thus it is foreseen to image additional samples at this the micrometer scale. This spatial resolution should enable the identification of micro-cracks at the expernse of getting a smaller field of view.

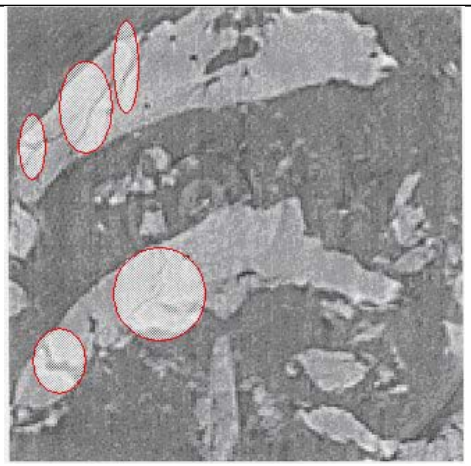

Figure 3 : micro-cracks are visible (circles) in the SR µCT images at 1.4 µm

References

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