ESRF	**Experiment title:** Bone vascularisation three dimensional analysis in the rat tibia	**Experiment number**: MD 141
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Report:

Context : Skeleton is a highly vascularised tissue uptaking ¼ of cardiac output. While the major role of bone vascularisation during the modelling process was well emphasised (bone growth, fracture reparation [1-2]), its role in the bone response to physiological or pathological stimuli that regulate bone remodelling is less understood ([3]). However, recent data obtained from in vitro studies suggested an intense cross-talk between bone and vessel cells ([4]). Our working hypothesis is that bone vascularisation, including angiogenesis and blood flow control, is involved in bone response to mechanical strain.

Aim of the work : The aim of our study was to show the feasability of imaging and measure the bone micro-vascularisation in adult rat tibiae using Synchrotron Radiation (SR) microCT at the ESRF. For this purpose, it was necessary to test several agent for vessel opacification and the injection proceedure.

Experimental method:

In order to better define the contrast agent, we injected with various agents a series of 4 month-old rats in veina cava after having thoroughly washed their vascular bed with heparinized PBS solution using intracardiac infusion right after sacrifice.

The following contrast products were used :

- Barium Sulfate (BS) (Micropaque Guerbet, solution orale, BS 100%) that was diluted at 30, 40, 50, 60, 70, 80%. Various diluents were used, PBS, Glycerol at various concentration (10, 20, 30, and 40%) in order to modify the viscosity of the injected product and prevent turbulent fluxes during infusion.
- Iodine (Telebrix) 100%
- Gadolinium 100%
- Bismuth chloride (70%)
- '(The options of lead or osmium were rejected because of toxicity and the major difficulties of safe handling inappropriate for this kind of experiment).

The solutions were injected using an electric syringe and various outputs wee tested (15ml/h, 30ml/h, 100ml/h, 150ml/h). In order to visualize the adequacy of the infusion we added to the injected solution a 12%

Vert lumière solution that colored the paws of the animals in bright green. Only the tibiae from rats correctly injected were submitted to SR micro-CT measurements.
In parallel, the same bone samples were submitted to 3D- microCT evaluation on a Scanco VivaCT in order to see whether it would be possible to image and quantitatively measure the vascular bed with conventional polychromatic microCT. Several energies were tested: 75 keV, 55 keV and 45 keV, in order to see which energy was optimal to differentiate bone from vascular signals.
All the solutions tested were into capillary tubes and linear coefficient was measured in the Scanco apparatus.
Results : SR microCT was performed on beamline ID19 at the 5µm scale (2048x2048 Frelon camera, pixel size on the detector 4.9µm). To reduce scan time we used the ondulator and energy of 23.3 keV. 1300 radiographs of 1700x800 pixels were acquired. The size of the reconstructed volumes were about 1300x1300x800 voxels.
No vascular profiles were observed with either gadolinium or iodine probably due to an quick diffusion of the products from the vessels to the tissues. The best contrast was obtained with SB (Micropaque) at 60% with a rather homogeneous penetration of the infused solution throughout the entire metaphysis. The 3D displays of the reconstructed volumes enable to identify the vascular signal. However, when simple thresholding was applied, the vessel network was quite discontinuous in small vessels and the imaging and quantitative
evaluation of the branching remains difficult. SB linear attenuation coefficient was 4 cm^{-1} in large vessels, bone varied between 3 and 5cm^{-1}, but bone and vessels showed overlapping peaks.

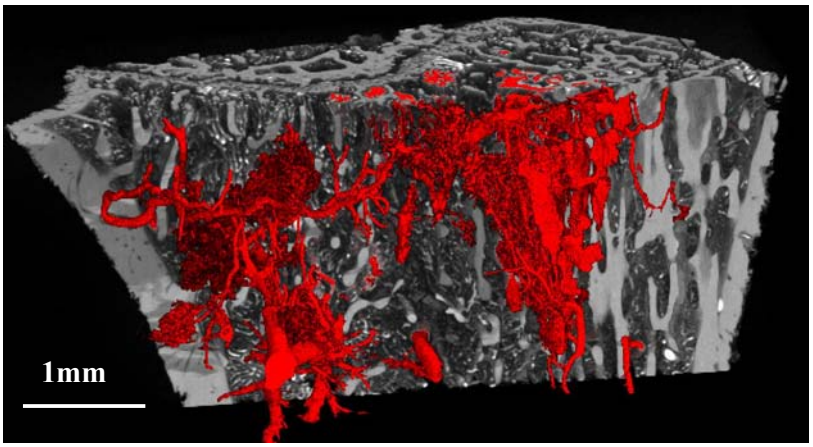

Figure 1 : Illustration of a 3D SR microCT image of vascularization in a rat tibiae showing simultaneaously bone (gray) and SB opacified vessels (red)

3D conventional microCT with the ScancoVivaCT showed that vascular bed was better observed at 70% of SB (45 KeV). SB linear attenuation coefficient was 8cm^{-1} in big vessels while that of bone varies from 3.8 to 4.8cm^{-1}. However, in small vessels the local coefficient was closer from that of bone and thresholding became difficult.
Conclusion : The injection and infusion techniques that was optimized in this work, seem appropriate in order to opacify the bone vascular bed. Baryum sulfate yields the optimal signal , thresholding between bone and vessel is easier with SR microCT than 3D microCT and due to better resolution ST microCT yields better images. Thus vascularisation quantization appears to be possible with SR microCT while it does not seem to be yet feasible with 3D microCT.

References

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