# Osteocytes number and volume in osteoporotic and in healthy bone biopsies analysed using Synchrotron CT: a pilot study

Ritter Z.[1], Staude A.[2], Prohaska S.[3], Brand R.[4] Friedmann A.[1,5], Hege H.C.[3], Goebbels J.[2], Felsenberg D[1].

1 Charité Universitätsmedizin Berlin
2 Federal Institute for materials research and testing, BAM
3 Zuse Institute Berlin (ZIB)
4 Visage Imaging, Berlin
5 Witten University, Faculty of Health, School of Dentistry, Department of Periodontology

Aims: Since in osteoporotic bones reduced osteocyte numbers appear to be related with the incapability of the bone to gain and maintain bone mass in the elderly, a pilot study aiming at establishing a methodology for measuring osteocytes by synchrotron radiation to quantify the number and volume was set. The major objective was to determine if the number of osteocytes, their volume, and their distribution along the cement lines are correlated with aging.

Materials and Methods: One biopsy from each of two groups of 20 healthy and 20 osteoporotic bone biopsies from the mandible scanned with a resolution of 20µm was selected in the same region (36) for this pilot study. The selection criterion was a difference ≥60% in the BV/TV parameter. The samples were fixed in 70% ethanol in tailored containers adapted for the measurement requirement at BESSY aiming at avoiding motion artifacts due to the high sensibility of the CT. The raw data were converted into image data using a reconstruction algorithm developed at BAM. The measurement time was approximately 24 hours per sample using an isotropic resolution of 2.174 µm. Two stacks were required per sample to measure each biopsy with diameters of two millimeter and 1 cm length. The image analysis was performed using Amira. After segmentation, a binary matrix containing only the osteocytes surfaces was obtained. Using a map of the voxel topology on this matrix, vertices of each osteocyte and in case of enlarged osteocyte surfaces a centerline with two vertices was determined. The number of the osteocytes was then calculated as the difference between the number of vertices and centerlines. To test this simple and reproducible method for calculation of the osteocytes number, it was compared to a manual counting of the osteocytes number in a minor and more manageable bone region. The total volume of the osteocytes was compared additionally.

Results: The healthy bone presented a larger number of osteocytes along the cement lines compared to the osteoporotic. Due to the high computational cost (each biopsy is approximately 50GB in size), the osteocytes were preliminary quantified in a selected region localized at the center of the biopsy. A difference of up to 64% in number and 55.3% in volume was found. However additionally, large regions of high attenuation coefficients between the cement lines in the osteoporotic samples were observed. To confirm these preliminary results, two additional biopsies have recently been scanned. A first reconstruction of their data showed the same behavior, but the osteocytes number and volume has not been completely quantified.

Discussion: Synchrotron CT using at least a resolution of 2.17µm is required to visualize and quantify osteocytes along cement lines. The interdisciplinary work between BAM, Charité and the Zuse Institute allowed a simple, reliable and reproducible method for quantifying and characterizing bone at different age stages. Apparently, not only a reduction in the number of osteocytes occurs in the elderly, but their size increases and they become sparely distributed in comparison with healthy bone biopsies from the mandible.