Elemental Imaging in Osteoarthritis

N. Zoeger[1], J.G. Hofstaetter[2,3], F. Meirer[1], C. Streli[1], P. Wobrauschek[1], S. Smolek[1], A. Maderitsch[1], P. Roschger[2] and G. Falkenberg[4]

[1]Vienna University of Technology, Atomic Institute, Stadionallee 2, 1020 Vienna, Austria
[2]Ludwig Boltzmann Institute of Osteology at the Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 4th Medical Department, Hanusch Hospital, Vienna, Austria
[3]Department of Orthopaedics, Vienna General Hospital, Medical University of Vienna, 1090, Vienna, Austria
[4]Hamburger Synchrotronstrahlungslabor HASYLAB am Deutschen Elektronen-Synchrotron, Notkestr. 85, 22603 Hamburg, Germany

Osteoarthritis (OA), the clinical syndrome of joint pain and dysfunction caused by joint degeneration, affects more people than any other joint disease. The incidence of osteoarthritis rises precipitously with age; as a result, the prevalence and burden of this disorder is increasing rapidly. The histological disease pattern of OA in human joints include fibrillation of articular cartilage, clefting, and cartilage loss. Besides cartilage lesions, a thickening of subchondral bone as well as a tidemark duplication is observed in OA. The appearance of multiple tidemarks indicates additional cartilage calcification and it is believed to be a sign of increased turnover in the affected joint. To date very little is known about the role of trace elements in normal articular cartilage and subchondral bone and even less is known about their role in diseases such as osteoarthritis. In a recent study [1] we could demonstrate that the formation of tidemarks is accompanied by an accumulation of the trace elements lead (Pb) and zinc (Zn) at this zones of calcification in joint bones with no macroscopical signs of OA. The purpose of this study was to determine the elemental distribution, with special emphasis on Zn and Pb, in articular cartilage and subchondral bone from human femoral heads with different degrees of OA.

All measurements have been carried out using the confocal μ-XRF set up at beamline L [2]. For optimized excitation conditions the Ni/C multilayer was used and the primary photon energy was tuned to 18keV. In order to minimize self absorption effects in the sample and to utilize data matching with backscattered electron (BE) images surface near fluorescence scans (detection volume for Ca just underneath the front surface of the sample) have been performed. Since the resolution of the system has been determined to be 17 x 20 x 15 μm^3 (depth x lateral x height) for an incident photon energy of E=9.7 keV step sizes for scanning were chosen to be 10μm in each direction. Analysis time per pixel was typically set to 5-7s. Elemental maps were obtained using the μXRF software package installed at the beamline.

Samples have been prepared as five-millimetre-thick sections which were cut perpendicular to the articular surface from the superior, weight bearing region of the femoral head. They were fixed in 70% ethanol, dehydrated through a series of alcohol, and embedded in polymethylmethacrylate (PMMA). The PMMA-blocks were polished using diamond suspension and carbon coated for BE imaging prior to μ-XRF analysis.

Results obtained from a 900 x 480μm^2 μ-XRF scan are shown in Figure 1. The analyzed cartilage-bone sample showed already advanced degeneration with the formation of fibrous articular cartilage and signs of subchondral bone thickening. However, despite the degeneration no alterations in the tidemark were seen. The region where the scan was performed was chosen to contain articular cartilage (AC), the tidemark (TM), calcified cartilage (CC) as well as subchondral bone (SB). From the comparison of the elemental maps with the BE image and the optical micrograph it can be concluded, that Ca is almost homogeniously distributed in zones of CC and SB with slightly elevated intensities in CC. Additionally a lower content of Ca was detected in zones of new bone formation which is in good agreement with the darker gray levels in the BE image. An almost similar distribution could be detected for Sr, but showing constant intensities in CC. Additionally increased Sr intensities were found in a zone of new bone formation (upper right corner of Fig.1). Zn and Pb show a strong accumulation at the TM. Whereas for Pb this is the only zone of increased intensities Zn could also be found at cement lines between bone packets of

different age, indicated by different gray levels in the BE images. These findings are in accordance with the results obtained in non-arthritic bones. Strikingly, elevated Zn intensities were detected at the outer border of the fibrous articular cartilage (AC) where no mineral is present. This phenomenon was not be seen in normal samples. Zn not only plays a critical role in bone mineralization. Numerous enzymes and proteins contain Zn and it is possible that there is an accumulation of zinc-containing enzymes in the surface region of heavily degenerated articular cartilage. However, what these proteins are and what their role in osteoarthritis is needs yet to be determined.

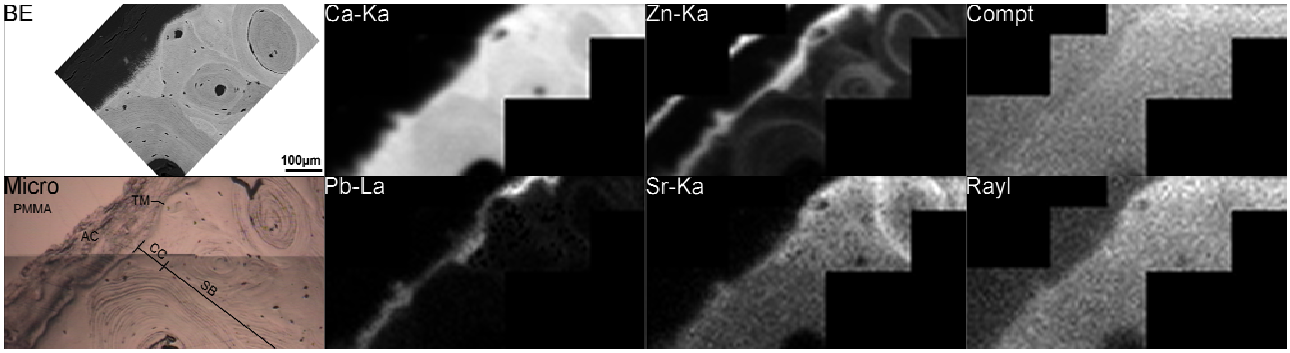

Figure 1: Backscattered electron (BE) image, optical micrograph (Micro) and elemental maps detected in a sample of arthritic hip head.

References

[1] N. Zoeger, P. Roschger, J.G. Hofstaetter, C. Jokubonis, G. Pepponi, G. Falkenberg, P. Fratzl, A. Berzlanovich, W. Osterode, C. Streli, and P. Wobrauschek, *Lead accumulation in tidemark of articular cartilage.* Osteoarthritis and Cartilage, 2006. **14**(9): p. 906-913.

[2] K. Janssens, K. Proost, and G. Falkenberg, *Confocal microscopic X-ray fluorescence at the HASYLAB microfocus beamline: characteristics and possibilities.* Spectrochimica Acta Part B: Atomic Spectroscopy, 2004. **59**(10-11): p. 1637-1645.