	Experiment title: **On the impact of implant-related particle exposure on immun and cellular response in human bone and bone marrow.**	**Experiment number**: MD1364
Beamline: ID21	**Date of experiment**: from: 13. 11. 2022 to: 17. 11. 2022 from: 21. 02. 2023 to: 23. 02. 2023°; 01.April 2023	**Date of report**: 01. September 2023
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Scientific background: The gradual release of metals from the non-articulating components of orthopaedic knee and hip implants (arthroplasties) is a potential source of adverse effects in the body. The wear debris from the articulating metal surfaces of the implants has been extensively studied, but the impact of metal release from the load-bearing (non-articulating) components has received less attention. Metal exposure may induce an immune response that causes chronic inflammation and implant loosening. The role of hypersensitivity reactions to metallic implant materials is still debated, despite the high prevalence of contact allergy to metals in the general population. We aim to investigate whether implant failure can be attributed to a delayed-type hypersensitivity reaction to implant metals in the bone marrow of affected patients. Our systematic investigation will provide new insights for early prediction of individual risk and possible intervention/prevention strategies.

ID21: SR-µXRF investigations on 10 µm thin sections from PMMA-embedded tissue samples were carried out at ID21. Experiments were performed using the in-vacuum scanning X-ray spectroscopy setup with an excitation energy of 7.8 keV. The X-ray beam was focused down to ~ 0.25 × 0.9 μm^2 (vertical × horizontal) with a flux of ~ 2 × 10^{10} photons/s. Acquisition time per pixel was 80 ms. Pixel size for collecting the XRF maps was set to 20 µm, 15 µm, 10 µm, 5 µm, 2 µm, or 1 µm depending on the size of the region of interest (ROI). Scans were acquired in continuous mode. All samples we planned to measure were investigated.

Results and summary: All samples that were scheduled for measurement using spatially resolved multi-element quantification by synchrotron-based X-ray fluorescence (XRF) at ID21 have been successfully measured. A total number of 240 scans were conducted on 34 samples. All spectra have been fitted and analysed, and elemental plots of the crucial elements have been generated. In the example shown in Fig.1, the HE histological image of one sample was registered within the reconstructed µCT volume of the same sample. The bone structures appear a bit different from the CT grayscale image (see Fig. 1b) because the histological image was somewhat brittle, and a few bone fragments were missing. The XRF analysis (Figure 1d-f) (XRF RoI indicated by pink box in Fig. 1c) revealed Ti and Co particles,

which, however, are not visible in both the histological image and the grayscale image from the µCT volume probably due to their sub-micrometer size. The XRF analysis also showed that Co and Cr are co-localized (elemental map of Cr not shown here).
Currently, the data is being analysed, compared, and correlated with immune-profiles and systemic metal values to provide a causal link between local immune cell activation, structural tissue changes and the metal exposure.

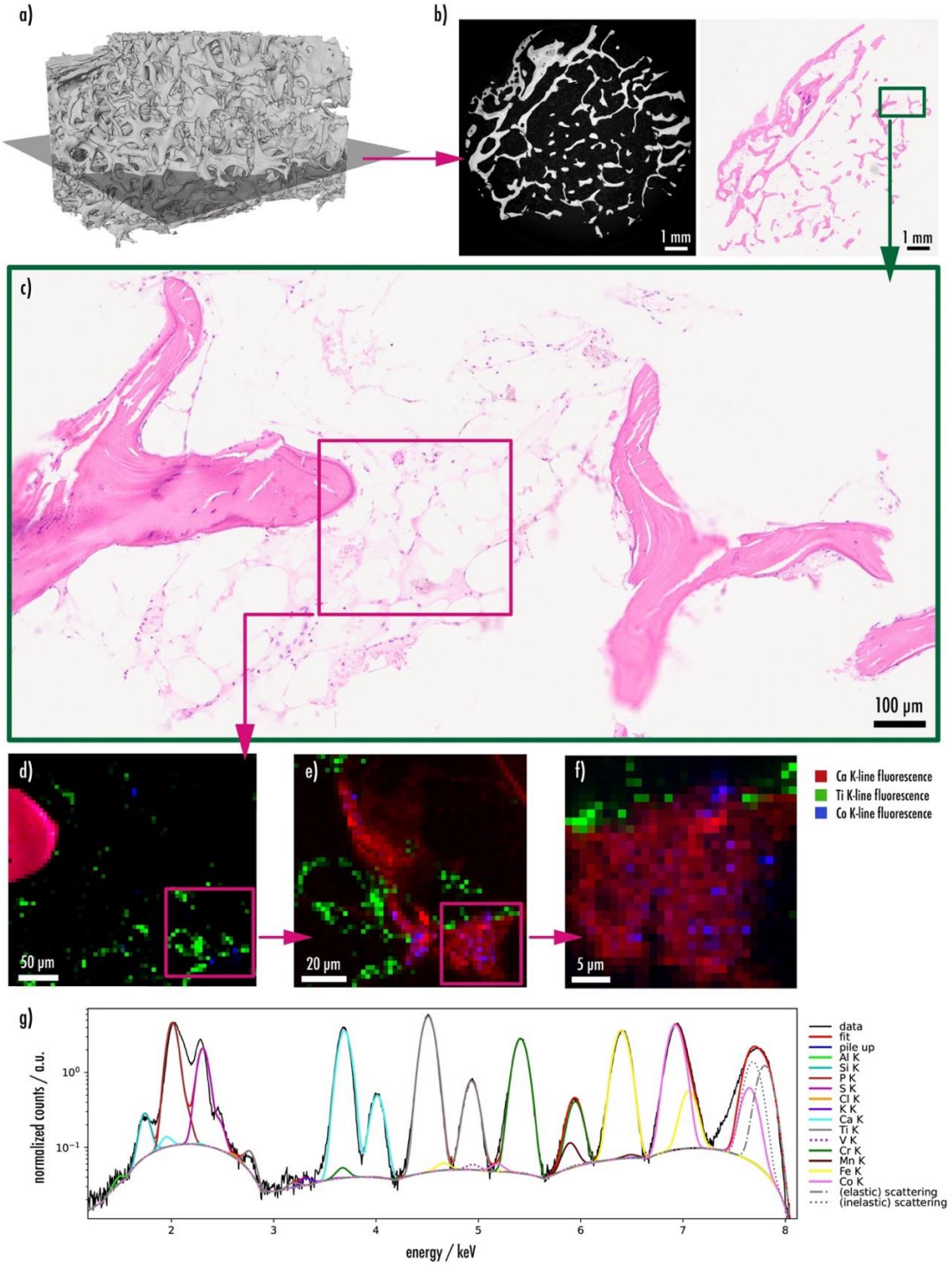

Figure 1 – multimodal analysis. a) rendering of µCT reconstructed volume. The black plane indicates the area from which the sections for histological staining analysis (see b and c) and for µXRF analysis were taken. b) grey value virtual section through the µCT volume as indicated by black plane. c) magnified image of the HE slide indicated by green box in b. The region of interest of the µXRF measurements is indicated by a pink box. d)-f) qualitative superimposed RGB images of the Ca (red), Ti (green) and Co (blue) net K-line fluorescence intensity. d) Overview scan with a 5 µm step size to locate areas for higher resolution scans. The area of d) was preselected with a fast scan (15 µm step size). e) high resolutions scan with a step size of 2 µm and f) 1 µm. g) fitted sum spectrum of f). Implant-related elements of interest such as Ti, (V), Co, Cr, Mn, Fe were detected and revealed local accumulation of titanium and Co/Cr particles.