ESRF	**Experiment title:** On the underlying mechanisms of chemical binding and accumulation in heart and brain tissue of Gadolinium of Gadolinium-based contrast agents.	**Experiment number**: MD1163
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Background

Contrast-enhanced magnetic resonance imaging (MRI) has the potential to replace angiographic evaluation of atherosclerosis. While studies have investigated contrast agent (CA) uptake in atherosclerotic plaques, exact CA spatial distribution on a microscale is still elusive. The purpose of this study was to investigate the micro-distribution of gadolinium (Gd)- and iron (Fe) oxide-based CA in atherosclerotic plaques of New Zealand White rabbits.

The study was performed as a *post hoc* analysis of archived tissue specimens obtained in a previous *in vivo* MRI study conducted to investigate signal changes induced by very small superparamagnetic iron oxide nanoparticles (VSOP) and Gd-BOPTA. For analytical discrimination from endogenous Fe, VSOP were doped with europium (Eu) resulting in Eu-VSOP. Formalin-fixed arterial specimens were cut into 5-µm serial sections and analyzed by immunohistochemistry (IHC: Movat's pentachrome, von Kossa, and Alcian blue (pH 1.0) staining, anti-smooth muscle cell actin (anti-SMA), and anti-rabbit macrophage (anti-RAM-11) immunostaining) and elemental microscopy with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) and synchrotron radiation µX-ray fluorescence (SR-µXRF) spectroscopy at ID21, ESRF. Elemental distribution maps of Fe, Eu, Gd, sulfur (S), phosphorus (P), and calcium (Ca) were investigated. Before carrying out SR-µXRF, adjacent sample sections were categorized by IHC into early lesion and advanced plaque using the pathologic plaque features defined by the American Health Association (control: n = 2, early lesion: n = 3, advanced plaque: n = 9).

ID21

SR-µXRF investigations on the sections adjacent to those used in LA-ICP-MS analysis were carried out at ID21. Experiments were performed using the in-vacuum scanning X-ray spectroscopy setup with an excitation energy of 7.3 keV. The X-ray beam was focused down to ~ 0.6 × 0.8 μm^2 (vertical × horizontal) with a flux of ~ 5×10^{10} photons/s (~ 180 mA SR current in multibunch mode). Acquisition time per pixel was 100 ms. Pixel size for collecting the XRF maps was set to 30 µm, 10 µm, 1–2 µm, or 0.5 µm depending on the size of the region of interest (ROI). Scans were acquired in continuous mode.

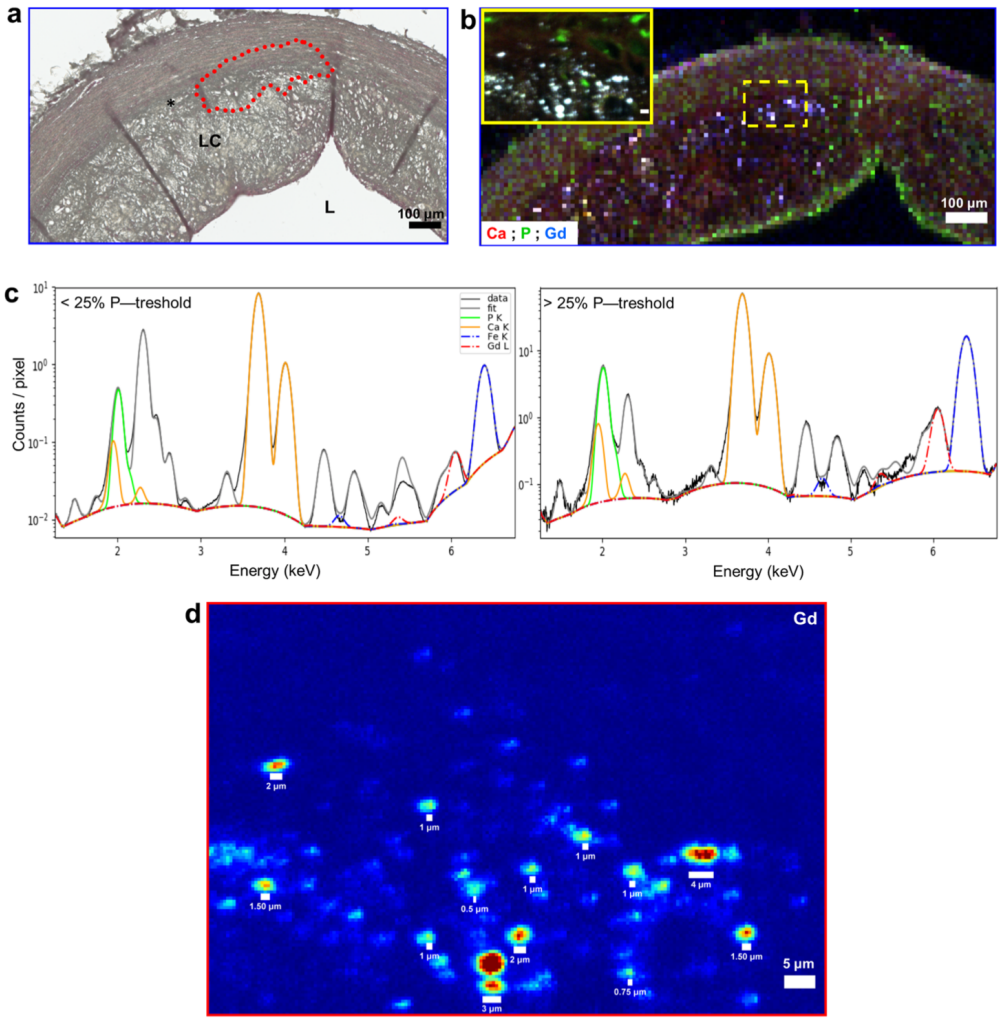

Figure 1 - Gd involvement in arterial calcification. a Micrograph of a von Kossa-stained advanced plaque ROI (× 15 magnification) reveals arterial calcifications, which are enriched at the intimomedial interface (asterisk, also indicated by the dashed red lines). Scale bar, 100 µm; L, lumen; LC, lipid core. b SR-µXRF analysis and RGB overlay of Ca, P, and Gd distribution at 10-µm and 0.5-µm resolution (ROIs are marked by yellow dashed lines). Scale bars, 100µm; ROI, 5µm. c Cellular uptake of Gd is investigated by analyzing the P distribution as a marker of cell membrane, ATP, or nucleic acids. P distribution maps at 0.5-µm resolution are segmented into two compartments by applying a 25 % threshold on the maximum of the P K-line fluorescence signal and comparing elemental concentrations in P-poor (G25% P-Threshold) or P-rich (925% P-Threshold) areas. Corresponding spectral deconvolution displaying colocalizing elements are normalized by the number of pixel of each region, thus giving an averaged spectrum for each region. The amplitude of the peak corresponding to the respective element is scaled by the amount of atoms being probed by the X-ray beam. In contrast to P-poor areas with G 1 mM, 62 mM, and 87 mM of Gd, P and Ca, respectively, P-rich areas contained 92mM, 779mM, and 789mM of Gd, P and Ca, respectively. Since P-rich compartments mark the cells or indicate close proximity to the cells, Gd could have been taken up by the cells, presumably by those that undergo calcified apoptosis, or may be involved in extracellular mineralization through complexation with P and Ca. d Size distribution analysis shows Gd-rich hotspots ranging from a few micrometers to submicrometers, which corroborates with von Kossa-stained calcified deposits, and indicates Gd involvement in arterial calcification.

Results and summary

Immunohistochemistry characterized atherosclerotic plaque pathomorphology. Elemental microscopy showed S distribution to match the anatomy of arterial vessel wall layers, while P distribution corresponded well with cellular areas. LA-ICP-MS revealed Gd and Fe with a limit of detection of ~ 0.1 nmol/g and ~ 100 nmol/g, respectively. Eu-positive signal identified VSOP presence in the vessel wall and allowed the comparison of Eu-VSOP and endogenous Fe distribution in tissue sections. Extracellular matrix material correlated with Eu signal intensity, Fe concentration, and maximum Gd concentration. Eu-VSOP were confined to endothelium in early lesions but accumulated in cellular areas in advanced plaques. Gd distribution was homogeneous in healthy arteries but inhomogeneous in early and advanced plaques. SR-µXRF scans at 0.5µm resolution revealed Gd hotspots with

increased P and Ca concentrations at the intimomedial interface, and a size distribution ranging from a few micrometers to submicrometers.

Eu-VSOP and Gd have distinct spatial distributions in atherosclerotic plaques. While Eu-VSOP distribution is more cell-associated and might be used to monitor atherosclerotic plaque progression, Gd distribution indicates arterial calcification and might help in characterizing plaque vulnerability.

Publication

The beamtime at ID21 went technically very well and the results have been published in:

Uca, Y.O., Hallmann, D., **Hesse, B., Seim, C.**, Stolzenburg, N., Pietsch, H., Schnorr, J. and **Taupitz, M.**, 2020. Microdistribution of Magnetic Resonance Imaging Contrast Agents in Atherosclerotic Plaques Determined by LA-ICP-MS and SR-μXRF Imaging. *Molecular Imaging and Biology*, pp.1-12.