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Application News

No. SCA-116-005

Mass Spectrometry Imaging, Inductively Coupled Plasma Mass Spectrometry

Complementary bioimaging of Gadofluorine P in myocardial infarction in mice

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■ Introduction

Magnetic resonance imaging (MRI) is a widely used imaging technique in the daily clinical practice. To enhance the contrast during this examination, several different contrast agents are available. Because of their excellent paramagnetic properties due to five or seven unpaired electrons, Fe^{3+}, Mn^{2+} or Gd^{3+} are mostly used. Since Gd^{3+} is toxic in the free form, this probe is administered as a complex with aminocarboxylic acids. While the most gadolinium-based contrast agents (GBCAs) distribute systemically, some target specific GBCAs are under investigation as well.

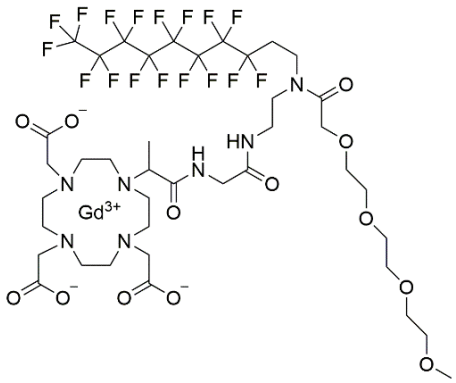

Figure 1: Structure of Gadofluorine P.

Gadofluorine P is one of these target specific contrast agent and shows high affinity towards the collagen-rich extracellular matrix (ECM), which is secreted in case of myocardial infarction (MI). The combination of two complementary bioimaging techniques is able to visualize this accumulation. Laser ablation with inductively coupled plasma mass spectrometry (LA-ICP-MS) is used to generate quantified images on an elemental level with high spatial resolution, while matrix- assisted laser desorption/ionization mass spectrometry (MALDI-MS) is used to validate the findings on a molecular level and provide further information regarding the distribution of for example phospholipids or heme *b*.

■ Materials and methods

Animal study

The animal study was performed in the research group of Prof. Moritz Wildgruber at the Institute of Clinical Radiology (University Hospital Muenster). Six weeks after an induced MI, the mice undergo MRI examination with injection of Gadofluorine P-solution as contrast agents. Afterwards the mice were sacrificed, the hearts were removed and snap frozen. Thin sections with a thickness of 10 µm were prepared with a cryomicrotome.

Standard preparation

For LA-ICP-MS analysis, matrix-matched standards based on gelatin were prepared for external calibration. Gelatin (10%w/w) was spiked with nine different concentrations ranging from 0 to 5000 µg/g Gd. Thin sections of the standards were also prepared with a thickness of 10 µm.

Sample preparation

For MALDI-MS imaging, the thin sections were placed on indium-tin oxide (ITO) slides. As a matrix is required, α-cyanohydroxycinnamic acid (CHCA) was deposited via sublimation onto the slide and the samples were afterwards recrystallized with 500 µl bidest. water and 50 µm methanol for 2.5 min.

Analytical conditions

For LA-ICP-MS analysis the ICPMS-2030 was coupled via a Tygon® tubing to the laser ablation system LSX-213 G2+ (Teledyne CETAC) equipped with the HelEX II cell and a Nd-YAG laser with a wavelength of 213 nm. Helium was used as flush and transport gas for the ablation cell. The ICP-MS 2030 was equipped with nickel sampler and skimmer.

Measurements were performed in collision gas mode with integration times of 100 ms for ^{31}P, ^{57}Fe, ^{66}Zn, ^{158}Gd and ^{160}Gd. For the calibration, 10 lines for each standard were analyzed with the same conditions as the sample (table 1).

Table 1: Experimental conditions used for LA-ICP-MS.

Laser ablation	**LSX-213 G2+**
Spot size	15 µm
Scan speed	30 µm/s
Shot frequency	20 Hz
Cell gas flow	0.8 l/min
ICP-MS	**ICPMS-2030**
Plasma power	1.2 kW
Sampling depth	5.0 mm
Plasma gas	9.0 l/min
Auxiliary gas	1.10 l/min
Carrier gas	0.45 l/min
Cell gas	6.0 ml/min
Cell voltage	-21 V

The MALDI-MS experiments were performed with the iMScope TRIO equipped with an ion trap – time of flight (IT-TOF) mass analyzer. Positive ion mode and a mass range from 700 to 1200 were selected. Additional experimental conditions can be found in table 2. The matrix was deposited with the iMLayer™ for 20 min.

Table 2: Experimental conditions used for MALDI-MS.

MALDI	**iMScope TRIO**
Spot size	40 µm
Sample voltage	3.5 kV
Detector voltage	1.9 kV
Accumulations	3
Laser shots	500
Laser frequency	1000 Hz

■ Results

LA-ICP-MS

External calibration with matrix matched standards showed a linear correlation for a concentration range up to 5000 µg/g with a correlation coefficient R^2 of 0.997. For the used spotsize of 15 µm a limit of detection (LOD) of 43 ng/g Gd based on ^{158}Gd was determined and the limit of quantification (LOQ) based on the same isotope was 140 ng/g Gd (calculations based on Boumans [1]).

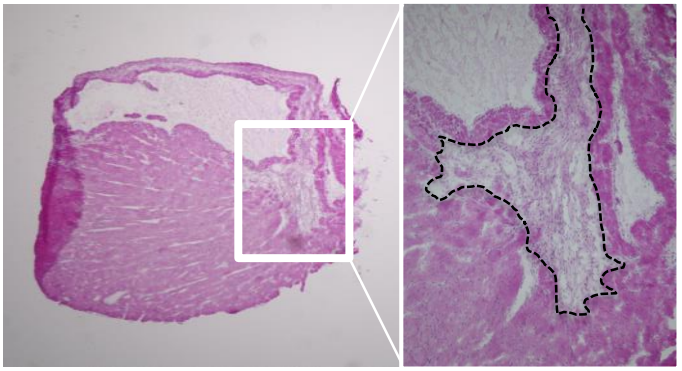

Figure 2: H&E staining of mice heart tissue section.

Hematoxylin and eosin staining was performed to detect the area of myocardial infarct (marked with a black line) on a parallel thin section shown in figure 2.

Figure 3 shows the microscopic images (a. and b.) of the two analysed thin sections. With LA-ICP-MS (c.), a homogenous distribution of the Gd in the healthy myocardium with an average concentration of about 50 µg/g was detected. The infarct region contains two times higher Gd concentrations of about 110 µg/g with maximum values up to 370 µg/g.

Higher Gd concentrations can also be found in the ventricle due to the intravenous administration of the contrast agent.

These distributions can be verified with MALDI-MS imaging (d.). In this experiment, only the protonated ligand of Gadofluorine P instead of the intact complex could be detected (e.). The main peak (*m*/*z* 1168.39) was used to create the image, which showed good correlation to the Gd distribution determined with LA-ICP-MS. Highest intensities of the molecular probe were found in MI and ventricle regions, whereas healthy myocardium shows low and homogenous intensities.

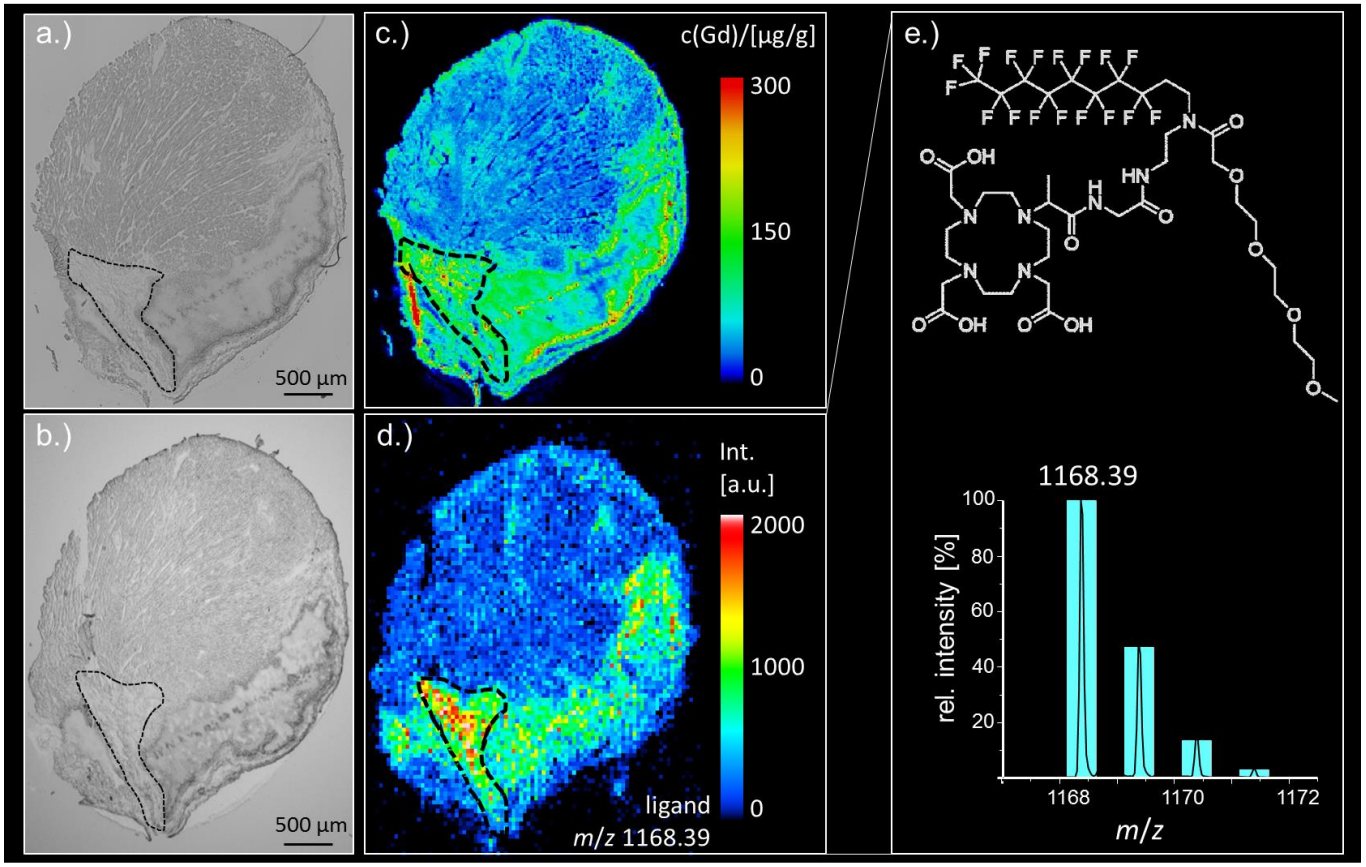

Figure 3: Microscopic images of the two parallel sections (a. and b.), quantified distribution of Gd determined with LA-ICP-MS (c.), distribution of the ligand from Gadofluorine P (d.) as well as the structure of the ligand and the theoretical spectrum (cyan bars) and the measured spectra (black line) with MALDI-MS (e.).

■ Conclusion

This application shows that the combination of an element selective (LA-ICP-MS) and a molecule selective (MALDI-MS) imaging technique is a powerful tool to visualize the distribution of target-specific gadolinium-based contrast agents in mouse heart tissue after myocardial infarction.
High spatial resolution and quantification were achieved by means of LA-ICP-MS and the distribution was verified on a molecular level by means of MALDI-MS.

■ References

[1] P.W.J.M. Boumans, Spectrochimica Acta 1991, 46 B, 641-665.

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