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Scaling down the bioimaging of metals by laser microdissection inductively coupled plasma mass spectrometry (LMD-ICP-MS)

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ABSTRACT

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been established as a powerful quantitative elemental imaging technique in routine mode for biological tissue with a spatial resolution of 12–160 μm. Several applications necessitate an improved spatial resolution of LA-ICP-MS at the low micrometre scale and below. To achieve the improvement of spatial resolution of LA-ICP-MS we created a new experimental arrangement by coupling a laser microdissection system (LMD) used for laser ablation of tissue with a sensitive quadrupole-based inductively coupled plasma mass spectrometer for the subsequent analysis of ablated material. A flat laser ablation chamber made of glass was inserted into the LMD, fitted to the microscope slide with the specimen. The biological tissue fixed on the glass slide was ablated using the focused solid-state Nd:YAG laser of the LMD. The laser ablated material was transported by argon as carrier gas into the inductively coupled plasma of the mass spectrometer and analysed according to the mass-to-charge ratio. Using this novel LMD-ICP-MS arrangement, in initial experiments ion signals of $^{63}Cu^+$ and $^{65}Cu^+$ were measured from a 30-μm-thick cryosection impregnated with a droplet of a Cu solution. A spatial resolution of about 3 μm was obtained using the modified LMD system coupled to the ICP-MS. Laser-induced mass spectrometric measurements of metal distributions can be performed together with simultaneous inspection of the tissue section via the microscope of the LMD and be combined with other modalities of the LMD system. In future, a more powerful laser in the LMD apparatus will allow ablation down to the sub-micrometre scale to study the elemental distribution in small tissue sections.

1. Introduction

Metal ions and metalloproteins play a key role in many biological processes, in ageing and in the origin of neurodegenerative diseases like Alzheimer's, Parkinson's diseases and Wilson's syndrome. Abnormal metal-containing deposits such as beta amyloid plaques and Lewy bodies are the neuropathological hallmarks of Alzheimer's and Parkinson's diseases, respectively. Metal ions critically influence misfolding and toxicity of proteins such as beta amyloid fragments, tau and α-synuclein [1–6]. This exemplifies that in the biomedical sciences there is also a growing interest in quantitatively assessing total metal concentrations within small structures in native tissue in situ. Extra-neuronal objects of interest are atherosclerotic deposits within blood vessels and the fate of smoke and dust particles in the lung. In the pharmaceutical sciences, the broad field of controlled delivery systems like diverse microcrystalline suspensions or nanoparticles and target-specific multimodal probes containing lanthanides or nanoparticles involve a plethora of applications for microlocal element analysis at the lower micrometre and nanometre scales.

Element-specific imaging techniques like EFTEM (energy-filtering transmission electron microscopy) [7], scanning electron microscopy with energy-dispersive X-ray analysis (SEM-EDX) [8], micro-XRF (X-ray fluorescence analysis) [9] or SIMS (secondary ion mass spectrometry) [10–12], cannot be applied to a series of open questions from the medicine, whereas synchrotron radiation XRF (SXRF) [13,14] and proton-induced X-ray emission (PIXE) [1] have extremely restricted availability. Several of these element analytical techniques provide a low dynamical range and encounter difficulties in quantifying analytical data. Furthermore, these imag-

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ing techniques (except SIMS) cannot analyse isotopes, which are interesting for studying the kinetics of metal metabolism in diseases using isotope-enriched tracers or for the application of the isotope dilution technique.

Among the existing analytical techniques for studying the metal and non-metal distribution in tissue, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become established as a very efficient and sensitive trace, ultratrace, and surface analytical technique in life science studies [5,15–18]. Quantitative LA-ICP-MS imaging of native biological tissues (rodent and human brain to study the metalloarchitecture in brain sections and neurodegenerative diseases) has been established as a routine technique in the BrainMet laboratory at Forschungszentrum Jülich (http://www.brainmet.com) during the last five years [19–21]. For the quantification of elemental images homogenized matrix-matched laboratory standards have been prepared and employed. This technique enables the measurement of elemental distributions from the mg g^{-1} to ng g^{-1} range in 10–40 μm thick cryosections. The spatial resolution of bioimaging LA-ICP-MS using commercially available laser ablation systems with solid-state lasers (Nd-YAG lasers at 266 and 213 nm wavelength, e.g., from NewWave UP 266 and UP 213 or from Cetac LSX 200 or 213) varied between 200 and 50 μm and has been restricted to approximately 5–10 μm due to limitations in the laser optics of commercial laser ablation systems.

Two analytical strategies have been pursued in order to improve the spatial resolution. One uses the near-field effect in laser ablation at a nanometre-scaled tip of a silver needle – so-called near-field laser ablation (NF-LA) – that is combined to a sensitive double focusing sector field ICP-MS (ICP-SFMS). The principles and application of NF-LA-ICP-MS have been described and discussed elsewhere [22–26].

Another innovative technique at low micrometre and sub-micrometre resolution would be the enhancement of a commercial laser microdissection (LMD) apparatus with a powerful solid-state laser to a sample introduction (laser ablation) system for a sensitive ICP-MS. The aim of this paper is a proof of concept of extending an LMD to form a laser ablation system coupled to an ICP-MS for thin tissue section analysis on a glass substrate with increased spatial resolution compared to conventional LA-ICP-MS.

1.1. A novel LMD-ICP-MS tool for the quantitative bioimaging of metals in small biological specimens

Laser microdissection (LMD) is a microscope-based technique using a high-precision laser beam applied via the microscope objective to selectively isolate specific cell types, individual cells, or cell organelles from embedded, frozen or fresh tissue sections. LMD is utilized in life science areas like drug discovery, pathology, forensics, medical diagnostics, food and environmental analysis as well as in medical research [27]. One of the aims in the conventional microdissection mode is maximum preservation of the selected sample area [28]. High-precision instruments for laser microdissection, applied for analysis in the fields of proteomics, genomics, bioarrays and biochips, offer highly resolved observation optics in combination with the option of cutting out previously identified and defined areas of a sample using a highly focused laser beam guided precisely over the sample. Specimens are mounted on a special PET membrane (e.g., 1.4 μm thickness) slide or directly on a microscope (glass) slide and placed on the table of the LMD apparatus.

The new idea was now to use an LMD apparatus with a high spatial resolution as laser ablation system together with a sensitive ICP-MS for elemental and isotope analysis on tissue. An LMD system using inverted microscopes is well suited for insertion of a laser ablation chamber because of the open space on top of the microscope stage. Brain samples may be much larger than the field of view of a microscope, therefore an LMD system with a fixed laser beam (e.g., SmartCut Plus system from MMI Molecular Machines and Industries, Zurich, Switzerland) where the sample is moved with a motorized microscope stage is preferred to investigate large areas of interest. A flat laser ablation chamber has to be constructed and mounted directly onto the glass slide with the specimen and connected to ICP-MS. The microscope stage control software can easily be modified in such a manner that laser ablation can then be performed directly online together with a quantitative and time-correlated determination of the distribution of elements in sample areas by LMD-ICP-MS. Images can be obtained than via a defined laser ablation process of the analysed area (the "line scan modus" – ablation line by line). For further studies on metallomics, preferentially by LMD, a corresponding analogue area can be cut out and analysed. After tryptic digestion of the cut-out tissue, for example, biomolecular mass spectrometry is used to analyse the tissue in terms of the structural determination of metal-binding proteins and/or phosphoproteins.

Extending LMD to form a laser ablation system and coupling it to a sensitive ICP mass spectrometer was proposed by Becker and Salber [29]. The advantage of this experimental arrangement is that a high-resolution microscope of LMD with significantly better resolution and higher precision compared to those in commercial laser ablation (LA) systems allows us to observe and select the small specific brain structures which can be ablated by the focused laser beam in general with a spatial resolution down to the low micrometre scale and below.

2. Experimental

2.1. Insertion of a flat laser ablation chamber into the laser microdissection apparatus (LMD) and coupling to ICP-MS

Because of the pivotal device of this study an appropriate laser ablation chamber was constructed that fits the object holder on the sample stage of the LMD microscope. The photograph of the laser ablation chamber (schematic diagram, see on right side in Fig. 1) before and after the insertion into the SmartCut Plus LMD (MMI Molecular Machines and Industries, Zurich, Switzerland) is shown in Fig. 1 a and b, respectively. The rectangular laser ablation chamber was constructed of borosilicate glass (Duran®, Schott AG, Mainz, Germany) with the inner dimensions 50 mm × 20 mm × 4 mm. The individual 76 mm × 26 mm microscope glass slide (StarFrost, Braunschweig, Germany) containing the sample section served as bottom and was sealed using a silicon foil or vacuum grease.

The laser ablation chamber was coupled to a quadrupole-based ICP-MS with hexapole collision cell (XSeries2, Thermo Fisher Scientific, Bremen, Germany) using standard Tygon tubes. The basic experimental setup is illustrated in the figure of Appendix B.

In our experimental arrangement, a Nd:YAG solid-state laser of LMD for laser ablation of biological tissue was employed at a wavelength of 355 nm with a repetition rate of 5 kHz. This repetition rate is significantly higher compared to commercial laser ablation systems of 20 Hz. The 500 ps laser pulses and precise optics produce a beam spot size of 1 μm and below, providing exceptional cutting and/or laser ablation accuracy. The LMD control software permits microlocal analysis within predefined single points, straight lines, free-hand traced lines and in a raster modus using a set of parallels.

2.2. Measurement procedure of biological tissue

The sample selected for this preliminary experiment was a 30 μm thick native cryosection of a block of mouse brain