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An approach for quantification of platinum distribution in tissues by LA-ICP-MS imaging using isotope dilution analysis

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ABSTRACT

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has been revealed as a convenient technique for trace elemental imaging in tissue sections, providing elemental 2D distribution at a quantitative level. For quantification purposes, in the last years several approaches have been proposed in the literature such as the use of CRMs or matrix matched standards. The use of Isotope Dilution (ID) for quantification by LA-ICP-MS has been also described, being mainly useful for bulk analysis but not feasible for spatial measurements so far. In this work, a quantification method based on ID analysis was developed by printing isotope-enriched inks onto kidney slices from rats treated with antitumoral Pt-based drugs using a commercial ink-jet device, in order to perform an elemental quantification in different areas from bio-images. For the ID experiments ^{194}Pt enriched platinum was used. The methodology was validated by deposition of natural Pt standard droplets with a known amount of Pt onto the surface of a control tissue, where could be quantified even 50 pg of Pt, with recoveries higher than 90%. The amount of Pt present in the whole kidney slices was quantified for cisplatin, carboplatin and oxaliplatin-treated rats. The results obtained were in accordance with those previously reported. The amount of Pt distributed between the medullar and cortical areas was also quantified, observing different behavior for the three drugs.

1. Introduction

LA-ICP-MS elemental imaging of biological samples was first described by Wang *et al.* in 1991 [1], and since then the number of applications have greatly increased due to the emergent interest in tissue elemental distributions. LA-ICP-MS technique presents numerous advantages such as high sensitivity (typical limits of detection at the $\mu g\ g^{-1}$ range), wide dynamic range (up to 9 orders of magnitude), multi-elemental capability, molecule structure independent response and high spatial resolution in the lower µm range. However, the lack of certified materials prevents the validation of reliable elemental quantification by two-dimensional mapping of target isotopes; being only possible the method validation to a lesser degree by spike recoveries

In order to obtain comparable LA-ICP-MS images, internal standardization is required to normalize the acquired bio-images compensating sample matrix or instrumental drift effects. Several internal standardization methods have been reported, such as the employment of matrix elements as reference (as the ^{13}C signal) [2,3] or the deposition of polymeric layers on or beneath the tissue slices [4]. Recently, novel and robust internal standardization methodologies have been described including the coating of the sample surface with metallic films such as gold [5] or spiked inks printed with commercial ink-jet printers [6,7]. However, these approaches cannot provide quantitative information.

With respect to signal calibration by LA-ICP-MS, the main issue is how to introduce calibrants in the sample matrix avoiding elemental fractionation. The standards must match the sample matrix to reduce differences in non-stoichiometric effects during vaporization, transport of the ablated material and plasma ionization. To overcome this challenge, several quantification strategies have been described in the last years, such as the quantification via external calibration by employing matrix-matched standards and CRMs [8]. Herein, Becker *et al.* were the

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first to apply custom laboratory prepared standards for quantitative imaging by LA-ICP-MS [9]. In most cases, standards were made of tissue homogenate [10], gelatin [11] or synthetic polymers [12,13] spiked with known amounts of the elements under study. Moreover, the online addition of standard solutions to the ablation chamber has been investigated as a possible means for quantification [14]. However, this approach presents important drawbacks such as the decreased sensitivity (between 20% and 35%) due to the dilution of the ablated material, and elemental fractionation effects as consequence of differences between the matrix of the standards and the sample.

It is well known the high performance of isotope dilution mass spectrometry (IDMS) for quantitative elemental analysis, providing usually highly precise and accurate results. IDMS has been also reported for LA-ICP-MS quantification. Methodologies employing pressed pellets [15], online addition of the spiked standards [14,16] or deposition of polymeric thin layers containing the ID-spike onto polyester substrates [17] for the production of solid reference materials have been described in the literature. However, the accurate and easy application of IDMS to elemental quantification in bulk analysis is hampered for tissues imaging due to complicated sample preparation and risk of elemental fractionation effects in post-ablation additions.

In this work, a LA-ICP-MS quantification approach using IDA with spikes in solid state on tissue samples is evaluated. For this purpose, isotope-enriched inks will be printed onto kidney slices from rats treated with anti-tumoral Pt-based drugs using a commercial ink-jet device. Pt content in different areas of the kidney will be compared for three different drugs and related with their different nephrotoxic behavior. The proposed strategy is simple, could reduce the elemental fractionation. and take advantage of the dry plasma, which provides improved atomization and reduces the matrix effects. On the other hand, the use of liquid standards (spiked ink) has important advantages such as availability and easy handling (dilution or doping). The proposed methodology will be validated by means of depositing natural Pt standard droplets with a known amount of Pt onto the surface of a control tissue.

2. Materials and methods

2.1. Reagents

High-purity HNO_3 and HCl, as well as the standard solution of natural platinum (1000 mg L^{-1}) were purchased from Merck (Darmstadt, Germany). The isotopically enriched ^{194}Pt standard was supplied by Chemotrade GmbH (Dusseldorf, Germany). Isotopically enriched Pt-spiked ink was supplied by Proteome Factory AG (Berlin, Germany). Cisplatin, carboplatin and oxaliplatin were purchased from Sigma Aldrich Chemie (St. Louis, MO, USA). All solutions were prepared employing ultrapure water (Milli-Q Ultra-pure water systems, Millipore, USA).

2.2. Animal treatment and tissue slices preparation

Animal studies were conducted in the Medicine and Experimental Surgery Unit of the Hospital General Universitario Gregorio Marañón (HGUGM, Madrid, Spain) as is described elsewhere [7]. All the procedures were approved by the Ethic Committee on Animal Experimentation from the HGUGM and animals were handled and cared for in accordance with Directive 2010/63/EU and Spanish Royal Decree 53/2013 on the protection of animals used for experimentation and other scientific purposes. The dosages of the drugs administered are equivalent to clinical treatment protocols [18–20]. The drugs were administered dissolved in saline solution (0.9% NaCl, Braun Medical S.A, Barcelona, Spain) and injected intraperitoneally (i.p.) in a single dose, being 5 mg kg^{-1} body weight (bw) for both cisplatin and oxaliplatin and 64 and 100 mg kg^{-1} for carboplatin. Control rats were only injected with saline solution. 5 days after treatment (when the maximum renal damage has taken place) all rats were anesthetized and sacrificed. Both kidneys from each rat were perfused with ice-cold saline solution and quickly removed. Then, kidneys were frozen in liquid nitrogen and stored at −80 °C until their cryo-cutting with a commercial cryostat (Thermo Fisher Scientific, HM525 NX) into 4-μm sagittal sections, and mounted onto Superfrost Plus slides (Thermo Fisher Scientific, Braunschweig, Germany). Glass slides were kept at −80 °C until LA-ICP-MS measurements. In order to avoid H_2O condensation and preserve the spatial resolution of the species, samples were thawed in a desiccator connected to a vacuum pump for fast drying prior to each analysis.

2.3. Tissue printing with isotope-enriched inks

The printing process was performed as previously described by Moraleja et al. [7] Isotopically enriched ^{194}Pt-spiked ink was produced on request by Proteome Factory AG (Berlin, Germany). Sample printing was performed with the optimal conditions previously reported: four cycles at 50% of printing density. In order to calculate the concentration of the Pt-containing ink per area unit, nitrocellulose papers were printed in the same manner as tissues before and after the sample printing process. Then, five 23.7 mm^2 circles of each nitrocellulose paper were punched, digested with HNO_3: HCl (1:3), evaporated and finally reconstituted in 2% (v/v) HCl, being the isotopes ^{194}Pt, ^{195}Pt and ^{196}Pt further quantified by direct infusion ICP-MS.

2.4. LA-ICP-MS measurements

Isotopes ^{194}Pt, ^{195}Pt and ^{196}Pt were measured with a NWR213 laser ablation system (ESI, Fremont, USA) coupled to a sector field ICP-MS (Element XR, Thermo Fisher Scientific, Bremen, Germany) as is described elsewhere [7]. Both instruments were synchronized triggering the signal and He was used as carrier gas to transport the ablated material to the plasma (He flow rate 1 L min^{-1}). Complete ablation of the tissue sample was done using the following conditions: 37–40% increasing laser energy, and line scan distance of 80 μm. Other LA experimental parameters were: 20 Hz repetition rate, 150 μm s^{-1} scan speed, and 100 μm of spot size. The ICP-MS operating conditions were daily tuned using a reference glass slide for maximum ^{137}Ba and ^{232}Th intensity, and oxide ratio (ThO/Th) below 0.7%. Optimal ICP-MS parameters were typically 1350 W RF power, Ar plasma gas 16 L min^{-1}, Ar carrier gas 0.6 L min^{-1}, low resolution mode (m/Δm:300), dwell time 10 ms, and E-Scan scan type.

The analysis of the whole tissue sections (200 mm^2 approximately) required about 5 h. Raw data points were converted to pixels with MatLab software (MathWorks, Natick, Massachusetts) and exported to Origin 8.5 (OriginLab Corporations, Northampton, MA), where color-coded images were produced. Images integration was conducted by the software Image ImageJ (http://rsbweb.nih.gov/ij/).

2.5. Quantification using IDA

The content (o mass) of the Pt present in the sample was calculated using standard equations for IDA [21]. A 10 mg mL^{-1} solution of the isotope-enriched Pt standard was prepared from the raw material [22] and added to the commercial ink following the optimized protocol elaborated by Proteome Factory AG (Berlin, Germany). The ^{194}Pt printed concentration was determined by mineralization of printed nitrocellulose membranes as described above. All solutions and reagents were carefully weighed with a high precision balance able to read 0.00001 g (Sartorius). The isotope ratio ^{194}Pt to ^{196}Pt was used for IDA calculations. Mass bias correction factor was determined every day by direct infusion of 1 μg L^{-1} natural Pt standard solution. The exponential model was used for the correction of the mass discrimination.