Single-event tandem ICP-mass spectrometry for the quantification of chemotherapeutic drug-derived Pt and endogenous elements in individual human cells

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Abstract

Single cell – tandem ICP-mass spectrometry (SC-ICP-MS/MS) was used for the determination of the absolute amount of Pt (coming from exposure to various concentration levels of cisplatin as a chemotherapeutic drug) and five endogenous elements (P, S, Fe, Cu and Zn) in individual human cells of three different types – Raji, Jurkat and Y79. Optimum conditions were obtained by using a sample introduction unit transporting cell suspension containing approx. 5 × 10^4 cells per mL at a flow rate of 10 μL min⁻¹ to a nebulizer with narrow internal diameter (250 μm i.d.), mounted onto a total consumption spray chamber. Interference-free conditions were obtained in tandem MS mode (i) for P and S by pressurizing the collision/reaction cell (CRC) with O₂ and monitoring the PO⁺ and SO⁺ reaction product ions and (ii) for Fe by pressurizing the CRC with NH₃ and monitoring the Fe(NH₃)²⁺ reaction product ion. The quantification approach was validated by comparison of the absolute amounts of the target elements (in fg per cell) as obtained using SC-ICP-MS/MS with those obtained after acid digestion of approx. 2 × 10^6 cells and subsequent solution ICP-MS/MS analysis (“bulk” analysis). A higher Pt cell content was observed upon increasing the concentration of the cisplatin solution the cells were exposed to during 24 h. The Pt mass per cell (fg) increased linearly as a function of the cisplatin concentration, but
1. Introduction

Inductively coupled plasma-mass spectrometry (ICP-MS) has evolved into the most powerful tool for (ultra)trace multi-element analysis. Its high detection power, multi-element capabilities, wide linear dynamic range and high sample throughput also make it an attractive tool for the clinical lab. The occurrence of spectral interferences has long been considered the major disadvantage of the technique, mainly hampering the determination of target elements with a mass ≤81 amu, but sector field ICP-MS instruments capable of working at higher mass resolution [1,2] and quadrupole-based instruments equipped with a collision-reaction cell (CRC) [3] allow such interferences to be avoided or mitigated, also in the context of multi-element analysis. The introduction of tandem ICP-MS (ICP-MS/MS), in which a CRC is mounted in-between two quadrupole filters, thus offering double mass selection, has substantially advanced the capabilities for resolving spectral overlap by means of chemical resolution [4,5]. In chemical resolution, selective reaction between ions and gas molecules in the CRC are relied on, either to strongly suppress the contribution of the interfering ions to the total signal intensity at the original mass-to-charge (m/z) ratio of the target analyte ion (on-mass approach) or to convert the analyte ion into a reaction product ion that can be measured interference-free at another mass-to-charge ratio (mass-shift approach). In the latter approach, potential overlap at the “new” m/z ratio is avoided by the double mass selection in ICP-MS/MS.

Originally, ICP-MS was developed for analyzing homogeneous aqueous solutions and most instruments worldwide are still deployed for this purpose only. However, Degueldre et al. [6], were the first to report the use of ICP-MS instrumentation in the context of single-entity analysis for analyzing zirconia colloids in water. In this approach, also termed single-particle ICP-MS or SP-ICP-MS, every individual entity entering the ICP gives rise to a burst of ions. The signal intensity is monitored in a time-resolved mode with a sufficiently short dwell time. The number of such bursts provides information on the particle number density, while the intensity of each burst is related to the content of the element monitored in each individual particle and thus, its size (assuming the composition of the particles is known). Meanwhile, this SP-ICP-MS approach has already been widely used for studying engineered nanoparticles (ENPs) [7,8].

Soon, it was realized that this approach also held promise for single-cell analysis, although this presents a far greater challenge [9]. The composition of a cell is typically far more complex than that of an ENP, the content of the mineral elements is vastly lower and cells are larger and more fragile entities than ENPs. In single-cell ICP-MS (SC-ICP-MS), the works published so far can be divided into two main categories [10]. One category contains approaches developed and used for the monitoring of entities imported into the cells (e.g., nanoparticles, quantum dots, metallodrugs) after cellular exposure [11–13]. The second, often more challenging category, contains those works focusing on the quantification of endogenous elements in the cells [14–16].

As in single-particle characterization, a dilute suspension of cells is aspirated and cells are introduced as discrete entities into the ICP, thus giving rise to bursts of ions, and the corresponding short transient signals are monitored in a temporally resolved mode with a low acquisition time (preferably sub-ms) [17]. On condition that the sample introduction efficiency and the sensitivity are calibrated, the frequency of short transient signals provides the information on the cell number concentration, while the integrated intensity of each transient signal can be used for determination of the amount of the target element in the corresponding individual cell [18]. The added value of such approach with respect to acid digestion of a sample containing many cells and subsequent traditional solution ICP-MS analysis is that insight is obtained into the heterogeneity of the cells in suspension as the cells are analyzed as individual entities, instead of obtaining cellular information at an average (“bulk”) level only.

However, it needs to be noted that compared to the characterization of ENPs, which are robust and often consist of one or two elements only, single-cell analysis is more challenging. Cells are considerably larger and more vulnerable than ENPs. This is especially true for human cells, which have a size between 5 and 100 μm, most of them falling in the range of 5–30 μm [19]. The traditional sample introduction system used in ICP-MS consists of a pneumatic nebulizer mounted onto a spray chamber, where the role of the latter is to remove droplets with a size >10 μm to avoid instabilities in the ICP ion source. These droplets are removed via gravitational settling and impact on the walls of the spray chamber [20,21]. Self-evidently, this disqualifies the use of a traditional cyclonic or Scott-type spray chamber in the context of SC-ICP-MS. Additionally, as human cells are more fragile owing to their thin and soft plasma membrane, also the nebulizer needs to be adapted as otherwise a substantial fraction of the cells lose their integrity during the process of nebulization. Therefore, a sample introduction system that enables an efficient introduction of individual intact cells to the plasma, is a crucial part of the set-up for SC-ICP-MS [22,23]. Over the past couple of years, several sample introduction systems intended for single cell introduction were developed to meet the demanding requirements for SC analysis, although delivering intact cells into the ICP still remains challenging [24–27].

In this work, we have evaluated the use of an MVX-7100 μl workstation (Teledyne CETAC Technologies, USA) as a sample introduction system combined with an Agilent 8900 ICP-MS/MS (Agilent Technologies, Japan) instrument for single cell analysis, aiming at both quantification of several endogenous elements and of Pt present as a result of exposure of the cells to cisplatin as a Pt-
containing chemotherapeutic drug. Pt-based drugs such as cisplatin and oxaliplatin are widely used in cancer therapeutics, despite the occurrence of side effects [28–30]. Unfortunately, not all patients show the desired response to such treatment and chemoresistance can also develop during the treatment. As patient response cannot be predicted, further investigation as to responsiveness/resistance is required and single-cell measurement of platinum uptake by SC-ICP-MS can play a role in this context. As a proof-of-concept, in this study, three types of human cell lines were investigated at the single-cell level after having been exposed to various concentration levels of cisplatin. In addition, also the concentration of the endogenous elements P, S, Fe, Cu and Zn was determined using SC-ICP-MS as the cisplatin treatment can have an effect on the concentration of these elements in the cell, thus affecting cellular homeostasis and the various processes in which these elements are involved in [16,31]. Sulfur occurs in almost all proteins, while P occurs in DNA, RNA, ATP and phospholipids [32,33]. More than one-third of the human proteins (especially the numerous enzymes which catalyze biological functions in the cells) contain essential metals, such as Fe, Cu and Zn [34,35]. Stress may affect the homeostasis in cellular systems and in the context of this work, the major stress exerted on the cells was their exposure to cisplatin. As a result of the absence of spectral overlap, the signal intensities of Pt, Cu and Zn could be monitored in “no gas” mode (i.e. with a vented CRC). For P, S and Fe, accurate quantification was jeopardized by the occurrence of spectral interference, such that chemical resolution was deployed to generate interference-free conditions. All SC-ICP-MS results reported on were validated by comparing the average SC-ICP-MS results to the results obtained upon acid digestion of a cell pellet and analysis of the digest thus obtained using traditional solution ICP-MS.

2. Experimental

2.1. Cell cultivation, cell sample collection and cell viability assay

The Raji line was obtained by Pulvertaft from a Burkitt’s lymphoma of the maxilla of an 11-years-old male and consists of lymphoblast-like cells in suspension [36]. The Jurkat cell line was established from the peripheral blood of a 14 year old male by Schneider et al. and consists of T lymphocytes in suspension [37]. The Y79 cell line was isolated by Reid et al. by explant culture of a primary tumor from an eye obtained immediately after enucleation and consists of retinoblastoma cells in suspension [38]. RPMI 1640 medium supplemented with fetal bovine serum (10% for Raji and Jurkat, 15% for Y79), 100 μg mL⁻¹ streptomycin and 100 IU mL⁻¹ penicillin were used for cell cultivation. Sterile tissue culture flasks (Avantor®, USA) with 75 cm² culture area and equipped with a vented cap incorporating a 0.22 μm hydrophobic filter to allow gas exchange and minimize risk of contamination were selected as cell cultivation recipients. Cell suspensions were incubated in an incubator with 5% CO₂. When the cells were in logarithmic growth phase, 3 mM cisplatin stock solution (in sterile milli-Q H₂O) was added into the cell flasks to get final cisplatin concentrations of 5, 10 and 20 μM. Then, cells were collected 24 h after incubation and were washed three times with PBS (phosphate-buffered saline solution) to remove dead cells, cellular debris or dissolved Pt that may otherwise hamper SC-ICP-MS analysis.

For the purpose of single-cell analysis, the cells were fixed with 3% paraformaldehyde at room temperature for 15 min, then the cells were washed three times with PBS and subsequently stored in 70% ethanol at 4 °C until analysis. The use of a cell fixation approach, rather than the analysis of fresh cells, was selected due to the greater flexibility provided by the increase in cell stability during sample storage. Also, previous experiments with fresh cells carried out at A&MS-UGent during the optimization of the SC-ICP-MS setup (data not shown) pointed towards a lower TE in the case of fresh cells compared to fixed cells. These differences could tentatively be attributed to an improvement in cell robustness as a result of cell fixation that might be beneficial for sample introduction into the ICP. Although further research is required for establishing the best sample preparation approach for SC-ICP-MS analysis, all cells analyzed in this work were subjected to cell fixation, following the procedure described above. Prior to SC-ICP-MS/MS analysis, the ethanol was removed by centrifugation and decanting, and the cells were washed once using milli-Q H₂O. The cells were finally re-suspended in milli-Q H₂O at a cell number concentration around 5 × 10⁴ cells mL⁻¹ prior to SC-ICP-MS analysis. For bulk analysis, around 2 × 10⁶ cells were collected in vials and a cell pellet was obtained after removing the supernatant. The cell pellet was transferred into a TFA beaker using 1.5 mL of concentrated HNO₃. After reaction visibly ceased, 0.5 mL of 9.8 M H₂O₂ was added into the beaker. The closed beaker was heated at 110 °C for 18 h on a hot plate. The temperature was then decreased to 90 °C to evaporate the liquid until only a small drop remained in the beaker. The digested cell samples were re-dissolved in 0.35 M HNO₃ and this solution was subjected to solution-based (i.e. bulk) ICP-MS/MS analysis. All steps of the cell digestion protocol were carried out in a class-10 clean lab (Pico-Trace, Germany) to avoid contamination.

For the purpose of assessing the cell viability, the half maximal inhibitory concentration (IC50) of cisplatin was evaluated using a CellTiter-Glo® 2.0 Cell Viability Assay (Promega, USA). Cells (1 × 10⁴) were plated in each well of a Nunc™ MicroWell™ 96-well, Nunclon delta-treated, flat-bottomed microplate (Thermo Fisher Scientific, USA) with a volume of 90 μL per well and incubated for 72 h. Cisplatin was serially diluted in the appropriate medium to 10-fold the final concentrations. For each treatment condition, 10 μL was added to 4 replicate wells (final concentrations ranging from 0.1 to 200 μM) and plates were incubated for 24 h. The CellTiter-Glo® Cell Viability Assay was performed according to the manufacturer’s (Promega) instructions. Briefly, the plates and the CellTiter-Glo® 2.0 reagent were equilibrated to room temperature. The CellTiter-Glo® 2.0 reagent was added to each well in a 1:1 (v/v) ratio. Cell lysis was induced by placing the plates on an orbital shaker for 2 min. After allowing the luminescent signal to stabilize for 10 min, the luminescence was measured using a Synergy™ HTX multimode microplate reader (BioTek, USA). The IC₅₀ value was determined using the Graphpad Prism software. This was performed for 3 biological replicates. A flow chart of the experimental design of this work is shown in Fig. S1 of the supplementary material.

2.2. Chemicals

For the target elements Pt, P, S, Cu and Zn, 1 g L⁻¹ single-element standard solutions were used in the context of method development and for calibration purposes. P and S standards were from Merck (Germany), Cu, Zn and Fe standards from ChemLab (Belgium) and Pt standard from Inorganic™ Ventures (USA). Au single-element standard solution from Chemlab and citrate-stabilized AuNPs standard reference material with a particle diameter of 60 nm (SRM 8013) from the National Institute of Standards and Technology (NIST, USA) were used for determining the transport efficiency. In the context of bulk analysis, Ga (ChemLab, Belgium) and TI (Inorganic Ventures, USA) were selected as internal standards (final concentration: 5 μg L⁻¹). Cisplatin was obtained from AdipoGen Life Science (Germany). RPMI medium 1640 (1X) + GlutaMAX was from Merck (Germany), ThermoFisher provided the Gibco phosphate-buffered saline solution (PBS, pH = 7.2) that was used in the process of cell sample collection. The

parafomaldehyde and 70% ethanol used to fix and to store the fixed cells were purchased from Fluka AG (Switzerland) and ChemLab (Belgium), respectively.

2.3. Instrumentation

All measurements were carried out using an Agilent 8900 ICP-MS/MS unit (Agilent Technologies, Japan). An MVX-7100 μL work-station provided by Teledyne CETAC Technologies (USA), was used as autosampler. This setup enables an accurate introduction of a minimum volume of sample solution or suspension (down to 5 μL) at low sample introduction flow rate (down to 5 μL min⁻¹) into the ICP. High-efficiency sample introduction glassware was supplied by Glass Expansion (Australia) and consisted of a MicroMist HE U-series low-gas, high-efficiency concentric glass nebulizer (200 μL min⁻¹) and a full consumption, low-volume, on-axis spray chamber (see Fig. 1). A fittings kit was provided for connecting the outlet of the capillary of the MVX-7100 unit to the inlet of the nebulizer, avoiding dead volume and sample loss, while a MicroJet outlet of the capillary of the MVX-7100 unit to the inlet of the series low-gas, high-efficiency sample introduction glassware was supplied by Glass Expansion (Australia) and consisted of a MicroMist HE U-series low-gas, high-efficiency concentric glass nebulizer (200 μL min⁻¹) and a full consumption, low-volume, on-axis spray chamber (see Fig. 1). Operating parameters and data acquisition settings for the Agilent 8900 ICP-MS/MS unit are summarized in Table 1. Cell counting was performed by using a Marienfeld-Superior hemocytometer (Paul Marienfeld GmbH & Co. KG, Germany).

2.4. Data processing

The results were first visualized using the Single Nanoparticle Analysis Module of the Agilent MassHunter Workstation software. Subsequently, all the raw data of SC-ICP-MS measurements were exported (as .csv files) and processed using the in-house developed Hyper Dimensional Image Processing (HDIP) software, which was originally intended for processing of mass spectrometry data in the context of elemental mapping (imaging) using laser ablation (LA) - ICP-MS. This software contains a module which automatically identifies transient signal peaks randomly originating from discrete events (here: the introduction of a cell in the ICP), calculates their integrated signal intensity and documents the signal duration (additional details about data treatment using HDIP are provided elsewhere) [39–41]. Statistical analysis was performed using SPSS Statistics 26 software for Windows (IBM Analytics, Belgium).

3. Results and discussion

3.1. Method development

In a first stage, ICP-MS/MS methodologies were selected in view of interference-free conditions and the highest possible sensitivity. As a result of the absence of potential spectral overlap, the approach for the determination of Pt is straightforward with a vented CRC and quadrupole settings Q1 = Q2 = 195. The nuclides of the endogenous elements all show mass numbers <81 amu, thus their monitoring may be hindered by spectral interference. There are two stable isotopes of Cu, 63Cu and 65Cu, with relative isotopic abundances of 69.1% and 30.9%, respectively. While the monitoring of 65Cu was affected by spectral overlap, Cu could be determined interference-free with a vented CRC by relying on the signal intensity of the lower-abundant 63Cu nuclide (Q1 = Q2 = 65). Also for Zn, the highest abundant isotope 65Zn (relative isotopic abundance: 48.6%) could not be used due to spectral overlap when working with a vented CRC, but monitoring of 68Zn (relative isotopic abundance of 27.9%) provided a viable alternative. For achieving interference-free conditions for the monitoring of P, S and Fe, the CRC was pressurized with a reaction gas. For both P (31P, mono-isotopic) and S (major isotope 32S, relative isotopic abundance of 95.2%), O₂ was selected as reaction gas to convert the original anlyte ions into 31P16O or 32S16O reaction product ions, measured in mass-shift mode (Q1 = 31 and Q2 = 47 for 31P, and Q1 = 32 and Q2 = 48 for 32S) [42,43]. Fe has four isotopes, the most abundant one at mass number 56 (relative isotopic abundance of 91.7%). As the signals of all Fe isotopes are subject to spectral overlap with Ar-based polyatomic ions, also for this target element, chemical resolution was opted for. Hence, NH₃ was used as reaction gas, and 56Fe(NH₃)₂⁺ was selected as reaction product ion to be monitored (Q1 = 56, Q2 = 90) [39,44,45].

Additionally, the methodologies described above were also used in the context of bulk analysis. For quantitative element determination after acid digestion, external calibration was relied on as calibration approach (with standards containing 0, 0.1, 0.5, 1.0, 2.5, 5.0 and 10 μg L⁻¹ Pt, Fe, Cu and Zn and 0, 0.5, 1.0, 2.5, 5.0, 10 and 25 μg L⁻¹ P and S), while Ga and Tl were used as internal standards (final concentration: 5 μg L⁻¹) to compensate for potential matrix effects and/or signal instability.

The sample introduction system used for SC-ICP-MS/MS consisted of a MicroMist nebulizer (with 250 μm inner diameter) and a total consumption spray chamber specifically developed for use in the context of cell analysis. Nebulizer gas flow rate and make-up gas flow rate were tuned. Due to the small diameter of the central capillary in the nebulizer, the nebulizer gas flow rate (maximum: 0.45 L min⁻¹) is much lower than under standard conditions, which aids introduction of cells into the ICP without affecting the cellular integrity. Highest sensitivity was obtained for a total sample introduction gas flow rate (nebulizer + make-up gas) of approx. 1.05 L min⁻¹. Results show that the cell distributions were fully separated from the background distributions with combinations of 0.1 L min⁻¹ nebulizer gas + 0.95 L min⁻¹ make-up gas and 0.15 L min⁻¹ nebulizer gas + 0.90 L min⁻¹ make-up gas. However, the cell distribution was not entirely separated from that of the background when using other nebulizer/make-up gas combinations, such as 0.35 L min⁻¹ nebulizer gas + 0.7 L min⁻¹ make-up gas. Although a previous work showed that a similar combination (0.4 + 0.7 L min⁻¹) provides the best results for the introduction of 2.5 μm polystyrene microspheres when using the same sample introduction setup [40]. This can be attributed to the lower...
robustness of cells compared to polymer particles. Based on these results, it was concluded that a higher nebulizer gas flow rate has a negative effect on the cell integrity. Upon comparing introduction efficiencies (i.e., number of events detected relative to those expected on the basis of the cell number density and volume of sample introduced into the ICP), the combination of 0.15 L min⁻¹ nebulizer gas + 0.90 L min⁻¹ make-up gas provided a better efficiency (approx. 15%) than that of 0.10 L min⁻¹ nebulizer gas + 0.95 L min⁻¹ make-up gas (approx. 10%). Therefore, 0.15 L min⁻¹ nebulizer gas flow rate paired with 0.9 L min⁻¹ make-up gas flow rate was considered a viable setting to introduce intact cells into the ICP using this sample introduction setup, and was used in all further SC-ICP-MS/MS measurements. Furthermore, this nebulizer gas flow rate was found to be very close to that recommended by the manufacturer for SC analysis. Self-evidently, an introduction efficiency of approx. 15% is still far from that expected when using a total sample consumption introduction system, although it can be seen as fit-for-purpose for SC-ICP-MS/MS analysis of extremely fragile human cells.

### 3.2. Transient signals and integrated intensity distributions

The main difference between conventional bulk elemental ICP-MS analysis and SC-ICP-MS analysis is the data acquisition mode. Time-resolved ICP-MS with high temporal resolution allows for the monitoring of the transient ion plumes generated from single cells. High temporal resolution here requires the acquisition time per data point to be short enough to provide real-time dynamic information on what is actually happening in the ICP. The shorter the acquisition time is set, the higher the temporal resolution is. With a dwell time of 0.1 ms, a burst of ions as occurring upon a single cell is introduced into the ICP leads to a transient signal that is described by > 1 data point. In fact, durations of approx. 0.5 ms (i.e., 5 data points with a dwell time of 0.1 ms) are typically reported on in the literature for events resulting from the introduction of discrete entities [46]. A shorter acquisition time also leads to a higher signal-to-background ratio in those time windows actually corresponding to (part of) the burst of ions formed from a single cell [47]. As main disadvantage, the use of dwell times within the microsecond range requires the signals corresponding to every individual event to be reconstructed for appropriate integration. This fast data acquisition approach was used for the sequential monitoring of exogenous (Pt) and endogenous (P, S, Fe, Cu and Zn) element concentrations in Raji, Jurkat and Y79 cells that were treated with 0, 5, 10 and 20 μM of cisplatin. The cell suspension containing approx. 5 × 10⁶ cells ml⁻¹ was introduced at 10 μL min⁻¹ by the MVX-7100 workstation. The sample volume was limited to 25 μL and the signal of every analyte was monitored during 200 s in fast temporally resolved mode (0.1 ms dwell time). The low sample introduction flow rate improves the signal-to-background ratio (ionic background) without sacrificing the intensity of every single event determined by ICP-MS, also allowing for approx. 150 s of signal monitoring while consuming a very limited amount of sample only. Fig. 2 illustrates the time-resolved transient signal as monitored during 60 s with a dwell time of 0.1 ms for Raji cells exposed to different cisplatin concentrations.

### 3.3. Effect of the use of a reaction gas on the signal duration

In this work, chemical resolution was used to overcome the spectral interferences otherwise jeopardizing the determination of P, S and Fe at ultra-trace concentration levels. As outlined earlier, O₂...
was selected as the reaction gas for the determination of P and S and NH$_3$ for that of Fe. The reaction product ions, PO$^+$, SO$^+$ and Fe(NH$_3$)$_2^+$, could be measured interference-free by using a mass-shift approach. It is interesting to note that the signal durations of the spikes, each related to the introduction of an individual cell into the ICP ion source, differ substantially when using a pressurized CRC instead of a vented one. Fig. S2 of the Supplementary Material shows the average signal for all target elements as present in the three cell types — Raji, Jurkat and Y79 — studied. The signals of $^{195}$Pt$^+$, $^{65}$Cu$^+$ and $^{66}$Zn$^+$ were monitored in no-gas mode (vented CRC), while the other elements were determined based on the signals for the reaction product ions $^{31}$PO$^+$, $^{32}$SO$^+$ and $^{56}$Fe(NH$_3$)$_2^+$. While the transient signals show an average duration of ca. 600–700 ms (determined as full-width at 10% of maximum height) in the case of the atomic ions, those for the product ions typically last for 3.0–3.5 ms. This phenomenon is in agreement with what we reported in an earlier publication on the influence that various collision/reaction cell gases exert on the transient signal duration for different types of ENPs of various sizes [39]. The extension of the transient signal duration upon pressurizing the cell with O$_2$ or NH$_3$ can thus be attributed to interaction of the ion cloud with the gas in the CRC. This effect of using CRC technology on the signal duration of events generated upon introduction of a single cell needs to be considered prior to selection of appropriate dilution factors to avoid the occurrence of double events. Furthermore, event durations of approx. 3 ms suggest that the selection of the best suited dwell time for single-event ICP-MS analysis using CRC technology needs to be revisited aiming to find the optimum "event duration/dwell time" ratio. In this work, a dwell time of 100 ms was used in both vented and pressurized CRC mode, as P, S, and Fe are elements typically characterized by relatively high background signals and the use of longer dwell times would have a negative impact on the signal-to-background ratio. However, further research in this context is required to evaluate the possibility of using longer dwell times for single-event signals characterized by anomalously longer signal durations.

3.4. Quantification of element contents in single cells

For quantification of the element content in single cells, the integrated signal intensity corresponding to every individual single event detected needs to be converted into the absolute amount (mass) of the target element present in a single cell. The amount (mass) of the different target elements can be calculated following...
the same principle as that of single-particle ICP-MS, as described in equation (1):

\[ m_c = \frac{\eta \cdot F \cdot t_d \cdot I}{b} \cdot 6 \times 10^{4} \]  

(1)

where \( m_c \) (ng) is the mass of the element in the cell, \( \eta \) is the transport efficiency, \( F \) (mL min\(^{-1}\)) is the sample introduction flow rate, \( t_d \) (ms) is the dwell time, \( I \) (cps) is the integrated intensity for a single-transient signal or spike, and \( b \) (cps L mg\(^{-1}\) mg\(^{-1}\)) is the slope of the ionic calibration curve \([48,49]\). The transport efficiency (TE) was calculated based on the particle size method described by Pace et al. \([50]\) This method relies on the use of a nanoparticle (NP) reference suspension (56.0 nm AuNPs NIST SRM 8013) and ionic liquid standard solutions of the same target element (i.e., Au) at different concentration levels. By using this approach, the ICP-MS response for particles and ions, respectively, is determined and the TE can be calculated as the ratio between the slope obtained for the ionic calibration and the slope obtained for the particle calibration, as described in equation (2):

\[ \eta = \frac{R_{\text{ionic}}}{R_{\text{NP}}} \]  

(2)

where \( R_{\text{ionic}} \) is the ICP-MS response for ions (cps mg\(^{-1}\)) and \( R_{\text{NP}} \) is the ICP-MS response for nanoparticles (cps mg\(^{-1}\)). In this work, the TE was calculated to be approx. 60%. It needs to be noted that this TE is significantly higher than that determined in the case of human cells (approx. 15%), which can be attributed to the larger dimensions of human cells compared to ions and NPs and to their fragile structure. The limits of detection (LODs) were calculated as 3 times the standard deviation of the blanks divided by the slope of the calibration curves. The theoretical LODs were found to be 0.004, 0.4, 0.4, 0.02, 0.02 and 0.04 fg for Pt, P, S, Fe, Cu and Zn, respectively.

Quantification as outlined above was carried out for all of the elements in Raji, Jurkat and Y79 cells and the 4 exposure conditions. After appropriate calculation of the absolute amounts (mass) for every individual cell, the event frequency as a function of the absolute amount (mass) of analyte in a single cell was paid attention to. The frequency versus absolute amount (mass) of Pt, P, S, Fe, Cu and Zn within Raji cells after having been exposed to 5 \( \mu \)M cisplatin during 24 h is provided as an example in Fig. 5.

For validation of the methodology, cell pellets containing approx. \( 2 \times 10^6 \) cells were digested with HNO\(_3\) and H\(_2\)O\(_2\) and the digest thus obtained was subjected to bulk analysis by means of conventional solution ICP-MS. The results obtained — element concentrations and the standard deviations for Pt, P, S, Fe, Cu and Zn in Raji, Jurkat and Y79 cells exposed to different doses of cisplatin, are shown in Table S1 of the Supplementary Material. At a 95% level of confidence, no significant differences for any of the elements determined (Pt, P, S, Fe, Cu and Zn) were found between the results obtained by SC-ICP-MS/MS and those obtained via solution ICP-MS/MS bulk analysis (paired \( t \)-test, \( t < t_{\text{critical}} \)). The fact that the SC-ICP-MS/MS results show a higher variability compared to the bulk results should not be seen as a disadvantage, as this is the result of interrogation of every individual cell, thus providing information...
on the variability between cells. To illustrate this biological spread, Fig. 6 shows the boxplots for the absolute amounts (masses) of Pt, P, S, Fe, Cu and Zn in Raji cells exposed to 0, 5, 10 and 20 μM of cisplatin.

For all three cell types, the absolute amount (mass) of Pt per cell is increasing with increasing concentration of the cisplatin solution the cells are exposed to during 24 h. Fig. S3 of the Supplementary Material shows an example of the Pt mass distributions obtained for Raji cells exposed to three different cisplatin concentrations as obtained using SC-ICP-MS. It was found that the distributions become significantly wider upon increasing cisplatin exposure, which may indicate a non-optimal treatment at high doses. A comparison of the Pt cell content for the three cell types is provided in Fig. 7A. It needs to be noted that the high variability is a result of the interrogation of thousands of cells, thus representing the biological spread. However, statistically significant differences in the Pt mass per cell were found between different exposure concentrations and cell types, as indicated via ANOVA (F > Fcritical). As can be seen, the Pt mass per cell (fg) increases linearly as a function of the cisplatin concentration, but a higher Pt uptake was found in the case of Jurkat cells compared to the other cell types. To gain further insight into the different Pt uptakes, the cytotoxic effect of cisplatin on the Raji, Jurkat and Y79 suspended cells was assessed by evaluating the ATP content (see Fig. 7B). After 24 h of treatment, the Raji and Y79 cell lines showed little response, with a maximal decrease in metabolic activity at 200 μM of 23% and 15.5%, respectively, only. The metabolic activity of the Jurkat cell line however was inhibited by cisplatin treatment in a dose-dependent manner, showing an IC50 value of 11.1 ± 1.3 μM [51–54]. ABC (ATP-binding cassette) multidrug transporters, such as P-glycoprotein, have been associated with chemotherapy resistance [55] in a multitude of cancer types, including retinoblastoma [56] and Burkitt lymphoma [57]. Presence of multidrug transporters could potentially mediate cisplatin resistance and explain the lower Pt uptake of Raji and Y79 cells compared to Jurkat cells, although further studies using specific inhibitors, such as tariquidar, are needed to unravel their actual role [55]. Unlike the clear increase in the Pt mass per cell upon increasing cisplatin exposure, revealing whether the increase in the amount of Pt per cell is accompanied by changes in the amounts of the endogenous elements is more challenging. In the case of Raji and Y79 cells, this increasing level of Pt did not have a marked effect on the cell contents of the endogenous elements monitored. However, a trend towards a lower P and S absolute amounts (masses) per cell was noticed in the case of Jurkat cells (Spearman’s correlation was significant at the 0.01 level, r = –1.000). As indicated above, the Jurkat cell line was found to be highly sensitive towards cisplatin with low μM IC50 concentrations. The exposure to this metallodrug results in DNA-adducts causing DNA damage response and oxidative stress [58], which may exhaust the S-based glutathione redox cycle. Increased cellular stress may further affect DNA, protein and lipid synthesis, thus resulting in a decrease in the P and S contents upon increasing cisplatin exposure for this cell type. The relatively stable levels of Fe, Cu and Zn upon cisplatin exposure may indicate that those
elements are parts of biomolecules not significantly affected by acute exposure to platinum-based anti-cancer compounds. Since three types of human cells were studied using SC-ICP-MS/MS, principal component analysis (PCA) was used to evaluate whether the different cell types could be distinguished from one another based on the absolute amounts (masses) of the endogenous elements determined. In the PCA model, the first two principal components contain approx. 85% of the total data variance. As can be seen in Fig. 8, the three different cell types can be distinguished based on the groups generated in the PC2 versus PC1 score plot. The concentrations of the endogenous elements are lower in the Jurkat cells than in the two other cell types, while Raji and Y79 cells could also be distinguished from one another, mainly based on differences in their absolute amounts of P and Zn. Interestingly, potential changes in the metal(loid)s content induced by Pt-drug treatment are limited and do not seem to affect the groups resulting from PCA. This study thus suggests that different cell types could be distinguished from one another on the basis of the absolute amounts of endogenous elements present and demonstrates the potential of single-cell ICP-MS as a “metallo-fingerprinting” tool.

4. Conclusions

With a combination of a commercially available sample introduction set-up, intended for single cell introduction, and a tandem ICP-MS/MS unit, the absolute amounts of Pt (coming from exposure to cisplatin) and of 5 endogenous elements (P, S, Fe, Cu and Zn) were accurately determined in individual human cells of three types – Raji (Burkitt’s lymphoma), Jurkat (lymphoblast, T-cell) and

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Fig. 6. Boxplots for the absolute amounts (masses) of Pt, P, S, Fe, Cu and Zn in Raji cells exposed to 0, 5, 10 and 20 μM cisplatin concentrations. These boxplots compile the median, quartiles and minimum and maximum values. The individual dots are low and high potential outliers and the stars are high extreme values.
Y79 (Human retinoblastoma). Suspension (containing approx. 5 × 10^4 cells mL^-1) was transported to a concentric nebulizer with 250 μm i.d. central capillary at 10 mL min^-1 only, thus reducing the ionic background while not affecting the sensitivity for the “spikes” generated upon introduction of an individual cell into the ICP. Also, the short acquisition time of 0.1 ms contributed to the high signal-to-background ratio required to determine the target elements in individual cells, some of which present at < 1 fg cell^-1. The effect of spectral interference otherwise jeopardizing accurate determination of P, S and Fe was successfully avoided by operating the ICP-MS/MS instrument with a collision/reaction cell pressurized with a suitable gas and monitoring reaction product ions of the target nuclides at another mass-to-charge ratio (mass shift). The approx. 4-fold extension of the duration of the transient signal profile when using a pressurized collision/reaction cell did not hinder the accurate quantification of these target elements. The results indicated that Jurkat cells contain lower amounts of the endogenous elements determined than the Raji and Y79 cells. In all cell types, the amount of Pt absorbed increases linearly upon increasing the concentration of the cisplatin the cells were exposed to during 24 h. The higher Pt uptake of Jurkat cells compared to Raji and Y79 cells was found to be related with differences in chemo-responsiveness, as indicated via a cell viability assay. The results make clear that SC-ICP-MS/MS is a suitable tool to study and characterize discrete biological entities, thus contributing to a better understanding of cellular mechanisms in health and disease.

CRediT authorship contribution statement

Tong Liu: Methodology, Validation, Formal analysis, Investigation, Writing — original draft. Eduardo Bolea-Fernandez: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing — review & editing, Supervision. Christophe Mangodt: Investigation, Writing — review & editing. Olivier De Wever: Resources, Writing — review & editing, Funding acquisition. Frank Vanhaecke: Resources, Writing — review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The devices used — the MVX-7100 μL sample introduction unit, the single-cell glassware and the AT8900 ICP-MS/MS instrument — were made available by Teledyne Cetac Technologies, Glass Expansion and Agilent Technologies, respectively.

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Appendix A. Supplementary data

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References

[1] N. Jakubowski, L. Moens, F. Vanhaecke, Sector field mass spectrometers in ICP-

