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Application note

Application of the ARIS for high-resolution LA-ICP-MS 2D Pt-mapping for revealing cisplatin-induced nephrotoxic side effects

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Foreword

This application note illustrates the potential of the Aerosol Rapid Introduction System (ARIS), an add-on for Teledyne CETAC Technologies laser ablation systems, capable of further enhancing the aerosol washout in laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) setups. This application note focuses on high-resolution elemental imaging in a specific biomedical context. The sample type used in this work and the conclusions derived from the LA-ICP-MS mapping are described in greater detail in a paper by Van Acker *et al*.[1]

Introduction

Cancer in all its aspects and forms has a significant influence on people's lives, but thanks to numerous discoveries in various research fields, new therapies have been developed which enhance the patient's quality of living and survival rates. The quest for new chemotherapeutic agents is still ongoing; cisplatin (*cis*-diamminedichloroplatinum(II)) is a substance currently used to treat a variety of cancers, such as testicular, ovarian, cervical and colorectal cancer. One of the major drawbacks of cisplatin use is the occurrence of severe side effects, mainly in the kidneys. These side effects are dose-limiting and it is estimated that around one in three patients who undergo cisplatin chemotherapy suffers from nephrotoxicity.[1] As the pathways through which renal damage occurs remain poorly understood, there is a growing necessity for label-free, sensitive methods capable of visualizing the *in vivo* distribution of candidate chemotherapeutics. LA-ICP-MS is a micro-analytical technique, capable of performing spatially resolved elemental analysis. It is characterized by low limits of detection, a wide linear dynamic range, multi-element capabilities and minimal sample preparation. In this work, high-resolution LA-ICP-MS imaging was performed on a thin kidney tissue section of a *Macaca fascicularis*, treated with a pharmacological dose of cisplatin (2.50 µg g^{-1} body weight).

Experimental section

An Analyte G2 ArF* excimer-based 193 nm LA-system (Teledyne CETAC Technologies, Omaha, NE, USA) was coupled to a quadrupole-based XSeries-II ICP-MS unit (Thermo Fisher Scientific, Bremen, Germany) *via* the ARIS, a system developed at Ghent University and commercialized by Teledyne CETAC Technologies. The ARIS provides rapid aerosol washout, thus enhancing the sensitivity and sample throughput of the setup. This higher sensitivity enables a better spatial resolution, which is required for demanding biomedical applications like this one. The kidney tissue sections were cut at a thickness of 5 µm and were fixed in formalin and subsequently embedded in paraffin. Subsequent sections were stained with hematoxylin and eosin (H&E) and submitted to a histopathological study in order to search for potential renal damage induced by the administered dose of cisplatin. As the subsequent kidney tissue sections are morphologically very similar, the regions of interest (ROIs) identified during the histopathological study were selected on the unstained section and subsequently analysed via LA-ICP-MS. The instrumental parameters were tuned and optimized for low oxide

formation, low laser-induced fractionation and maximum sensitivity. The small regions of interest were ablated in line scanning mode with a circular laser spot of 3 µm diameter, 20 µm s^{-1} lateral scan speed, 60 Hz repetition rate, 3.04 J cm^{-2} laser output energy and carrier (He) and make-up gas (Ar) flow rates of 0.49 and 0.68 L min^{-1}. Furthermore, a larger region was mapped at lower resolution with a circular laser spot of 15 µm diameter in order to visualize the cisplatin distribution in a radial kidney segment, covering the renal cortex and medulla. The instrumental parameters applied, were 120 µm s^{-1} lateral scan speed, 50 Hz repetition rate, 4.72 J cm^{-2} laser output energy and 0.55 L min^{-1} He carrier gas flow rate.

Results

High-resolution brightfield micrographs taken from the kidney tissue sections were imported into the Chromium 2.2 software – the LA software suite provided with the Teledyne CETAC Technologies LA-systems – and subsequently aligned by using multiple alignment marks based on shared morphological features in the imported image and live camera view. This feature of the Chromium software allowed specific regions of interest on the section, selected based on an adjacent H&E stained image, to be targeted thus enabling LA-ICP-MS analysis at the desired position on the sample. Figure 1 shows the high-resolution brightfield micrograph with the rectangular regions of interest (1-3) indicated. By providing the high-resolution brightfield microscopic and LA-ICP-MS images side by side or overlaying them, adequate interpretation is facilitated. In Figures 1-1 and 1-2, two structures corresponding to the renal corpuscles are indicated. These structures are responsible for filtration of the blood, hereby removing water, electrolytes and small proteins, forming the primary urine which flows through thin tubules. Multiple functional segments of these tubules will reabsorb most of the water, electrolytes and glucose from the primary urine, ultimately forming urine as an end product. In the case of cisplatin, this substance is filtered out of the blood by the renal corpuscles together with the primary urine and should ultimately be excreted out of the body with the final urine. Unfortunately, partial reabsorption occurs in some functional segments of the tubules, such that cisplatin can accumulate in the epithelial cells of these tubules. From both LA-ICP-MS images, it can be concluded that no accumulation of cisplatin occurs inside the renal corpuscles, as the ^{195}Pt signal intensities are close to zero. The epithelial cells of the tubules light up in the elemental images. In Figure 1-3, a region is highlighted where multiple necrotic cells have been observed. These cells are visible in the LA-ICP-MS images as hotspots due to the high accumulation of cisplatin in these cells. Figure 1-4 shows the ^{195}Pt distribution in a radial segment of the kidney tissue section at a lower resolution than the previous images. The highest ^{195}Pt signal intensities are observed in the outside band of the kidney, which is called the cortex. It is in this region that the most important blood filtrations processes occur.

Conclusions

The highest cisplatin accumulation was observed in the renal cortex and hotspots correlated well with the presence of necrotic cells. (Sub-)cellular imaging was enabled by the use of the ARIS since this system provides a significant faster washout, resulting in a higher sensitivity and allowing the use of smaller spot sizes for higher resolution imaging.

References

1. Van Acker, T. *et al.* High-resolution laser ablation-inductively coupled plasma-mass spectrometry imaging of cisplatin-induced nephrotoxic side effects. *Anal. Chim. Acta* **945,** 23–30 (2016).

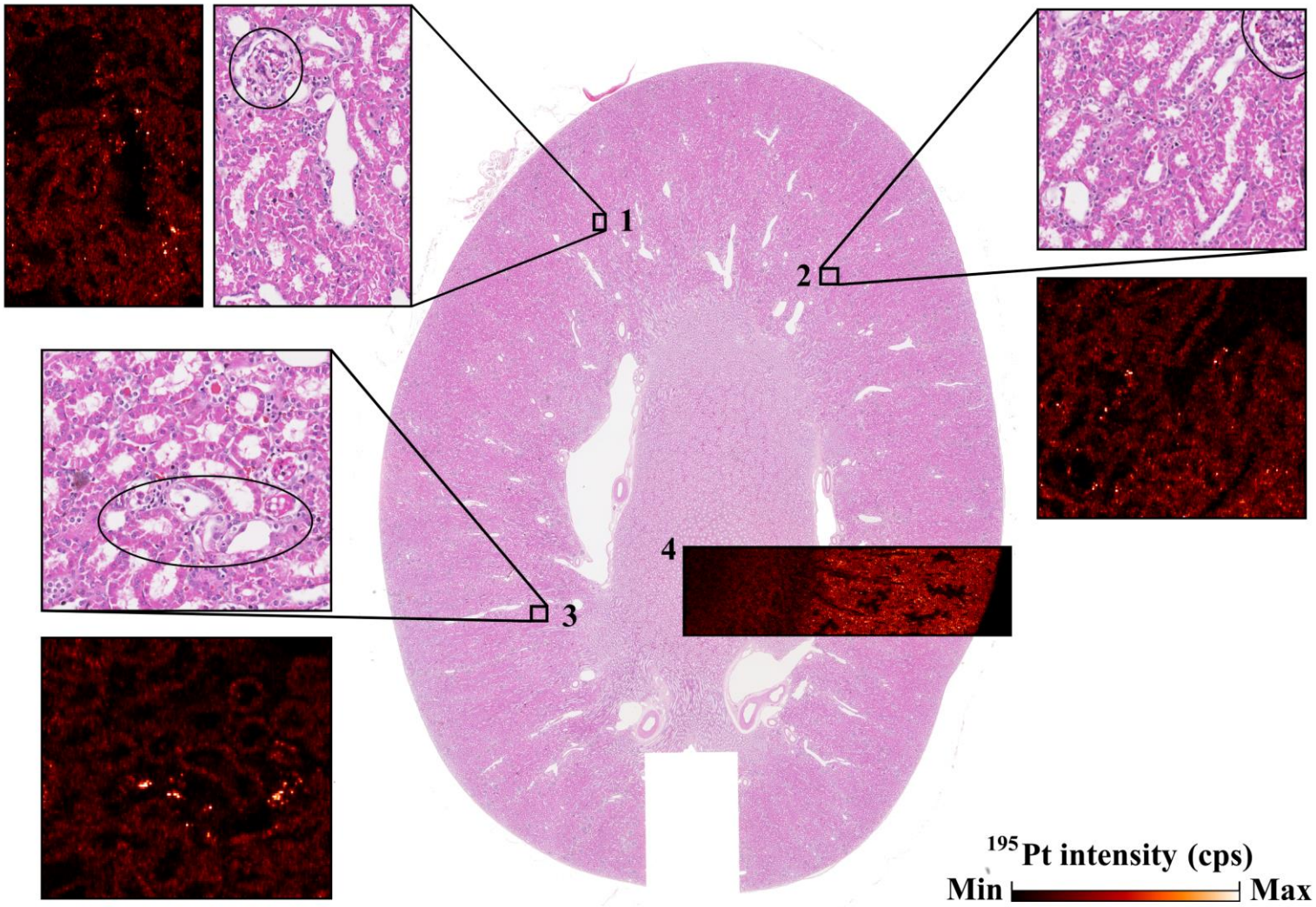

Figure 1. Overview brightfield micrograph of an H&E stained kidney tissue section and the selected regions of interest with corresponding LA-ICP-MS images displaying the ^{195}Pt signal intensity (1-3) and a radial segment ^{195}Pt distribution map (4).The minimum-maximum values for the ^{195}Pt intensity color bar are 0-200, 0-220, 0-180 and 0-5000 counts per second, respectively.