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Abstracts

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3. Speciation, Metalloids, and Biodistribution/Imaging Analysis

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T01 MULTI-ELEMENT ANALYSIS IN HUMAN CELLS USING SINGLE CELL ICP-MS. Tetsuo Kubota, tetsuo.kubota@agilent.com, Michiko Yamanaka, Agilent Technologies, 9-1 Takakura-machi, Hachioji-shi, Tokyo, Japan 192-0033; Risako Iida, Yu-ki Tanaka, Yasumitsu Ogra, Chiba University, Department of Toxicology and Environmental Health, 1-8-1, Inohana, Chuou-ku, Chiba-shi, Japan 260-0856

Many elements are essential for the biochemical processes in cells, and changes in the levels of these metals may disrupt natural cell processes. Single-cell inductively coupled plasma mass spectrometry (scICP-MS) directly quantifies the metal content within individual cells. ICP-MS has high analytical sensitivity and fast multi-element analysis capability, combined with the ability to handle very small sample volumes. These characteristics allow researchers to determine intracellular metal concentrations at the attogram (ag, 10^{-18} g)-per-cell level in environmental, life science, and clinical research applications, with minimal sample preparation.

Agilent 8900 triple quadrupole ICP-MS (ICP-QQQ) offers the benefit of tandem MS (MS/MS) operation, which effectively removes severe interferences on some elements of interest such as P and S. These elements are naturally present in cells at high level and can be used as internal standards to validate the scICP-MS method.

Using scICP-MS for cell samples cultured in various conditions, the impacts of those conditions on cells can be assessed. In this study, the uptake of Cd into cultured human myeloid leukemia cells (K562 cell line) was investigated. Zn and P were also measured in the same cell batch and the effect of Cd concentrations on Zn and P levels in cells was studied.

This study suggests that scICP-MS can be utilized for cytotoxicity assessment and for understanding the role of metals in cell processes.

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T02 MEASUREMENT OF PBMC CELLS INTRINSIC METALS CONTENT USING SINGLE CELL ICP-MS. Chady Stephan, chady.stephan@perkinElmer.com, PerkinElmer Inc., 501 Rowntree Dairy Road, Unit#6, Woodbridge, ON L4L 8H1 Canada; Magdalena Muszyñska, magdalena.muszynska@pepolska.pl, Pro-Environment Polska Sp. z o.o., 101 ¯wirki i Wigury Str., Warsaw, 02-089 Poland

Single cell inductively coupled plasma mass spectrometry (SC-ICP-MS) is an emerging analytical technique in the fields of environmental and biological sciences that enables the quantification of metals (ions or particles) on a single-cell basis.

Peripheral Blood Mononuclear Cell (PBMC) is any peripheral blood cell having a round nucleus. It is a population of blood cells that is composed mostly of lymphocytes and monocytes. The average diameter of PBMS cells is 7 µm. PBMC cells are easy to isolate and are widely used in research in the field of new drug and vaccine development in immunology, oncology and epidemiology. In this work, we compared intrinsic metals content in PBMC cells with the intent of understanding its relation to early identifications of diseases.

Material and methods

Cell isolation. PBMCs were isolated using Ficoll paque premium 1.084 (GE). Freshly collected blood was diluted with PBS and added into test tube containing Ficoll paque without disturbing the layers. Next the test tube was centrifuged at 1000 g for 10 minutes with the breaks off ensuring proper cell isolation. After centrifugation PBMC cells located in the phase between plasma and Ficoll layers were transferred to another test tube. PBMCs were washed twice with fresh PBS.

Cell counting. An aliquot of the washed PBMCs was diluted with ultrapure water and counted using a hemocytometer under light microscope.

Instrumentation. All measurements were performed on a NexION 2000 ICP-MS equipped with PerkinElmer Single Cell Sample Introduction Kit and Single Cell Micro DX Autosampler.

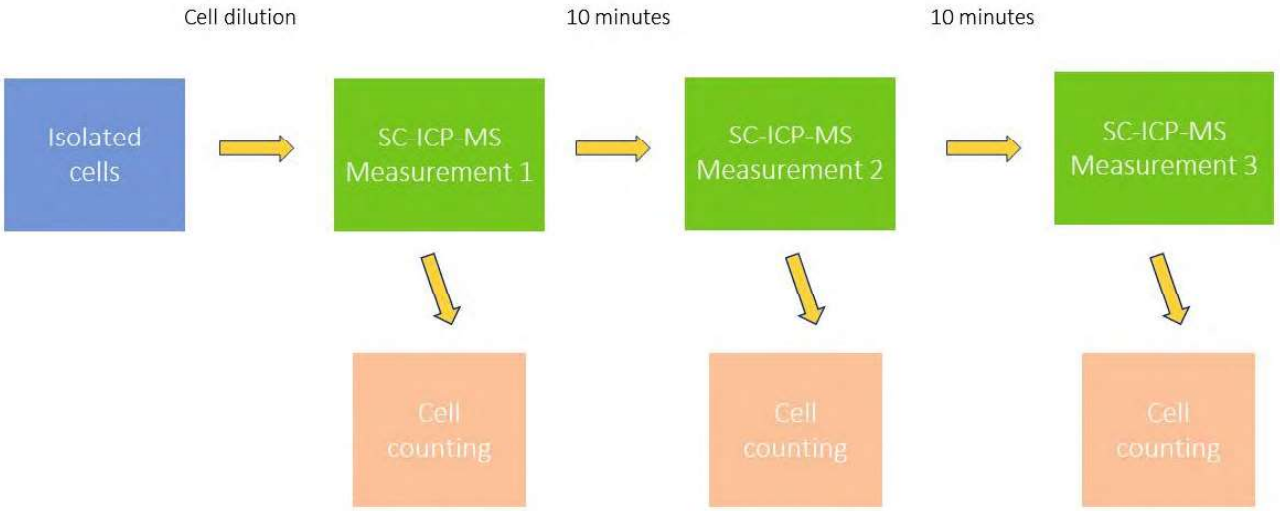

Figure 1. Scheme of the experiment.

Experiment

PBMCs were diluted with ultrapure water and measured exactly after the dilution. In PBMC cells population the intrinsic content of copper (Cu), iron (Fe), zinc (Zn) and magnesium (Mg) was measured. The same cell population was measured after 10 and 20 minutes. In each step, PCMCs were counted using hemocytometer [Figure 1].

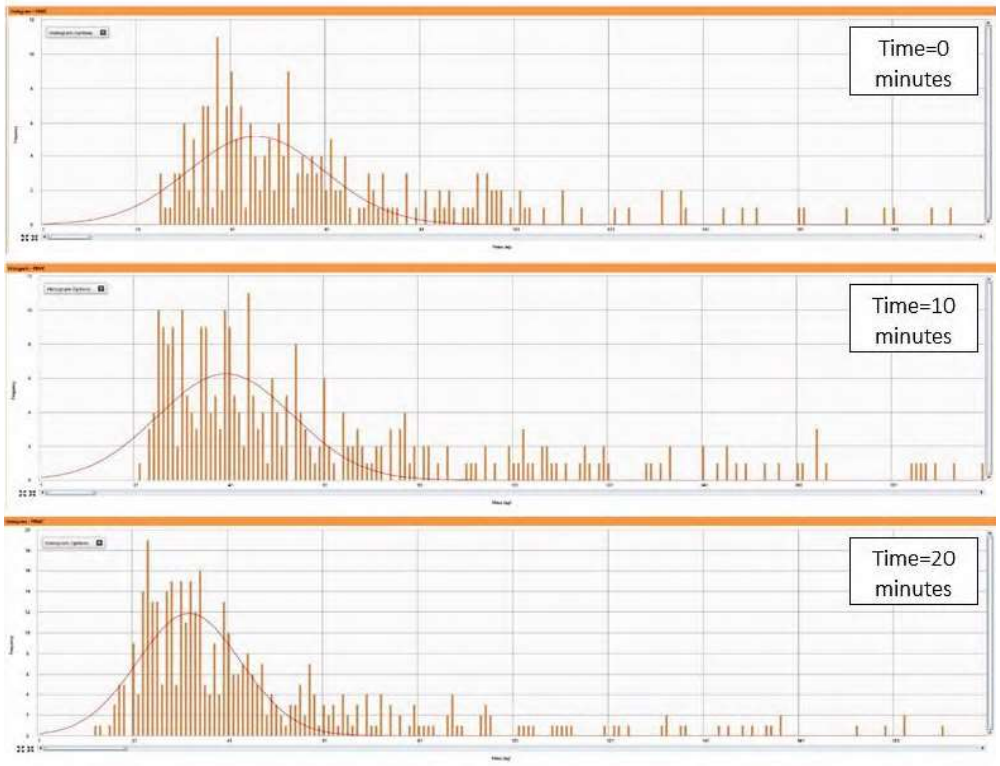

Figure 2. Result in iron distribution in PBMCs population

Results and discussion

The measurement of elements (Cu, Fe, Zn, Mg) content in PBMCs showed the possibility to analyze the distribution of intrinsic metals within this cells population. One of the interesting things

observed was the change of element distribution with time [Figure 2]. The distribution was shifted towards smaller masses with a simultaneous increase in the number of cells. The number of cells under the microscope decreased, so that we believe it was caused by cell breakdown when the cell parts were measured as an individual events. This observation would not be possible using classical ICP-MS measurement of metal content.

T03 ADVANCED IMAGING MODE FOR LA-ICP-TOFMS. Christoph Neff, cneff@inorg.chem.ethz.ch, Peter Keresztes Schmidt, Lena Bastian, Gunnar Schwarz, Bodo Hattendorf, Detlef Günther, Laboratory of Inorganic Chemistry, ETH Zurich, CH-8093 Zurich, Switzerland; Daniela Rubatto, Institute of Geological Sciences, University of Bern, CH-3012 Bern, Switzerland, and Paolo S. Garofalo, Dipartimento di Scienze Biologiche, Geologiche ed Ambientali, Università di Bologna, I-40127 Bologna, Italy

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) imaging showed significant improvement in recent years. Especially the use of the time-of-flight mass analyzer (TOFMS) and low dispersion ablation cells allow high lateral resolution, fast data acquisition, and multi-element detection [1-4].

In this work, we present an approach to control translation stage, laser, and ICP-TOFMS, which allows us to generate multi-element images with high speed, high sensitivity and direct link of the ablation position to the TOFMS signal. The setup permits a “hole drilling imaging mode” where multiple laser pulses are fired on the same sample position and are integrated [5]. The system allows also fast single pulse high spatial resolution imaging with edge-to-edge laser spots [1] and custom ablation area masks fitted to the sample structure. All these modes are used with synchronized laser ablation and TOFMS data acquisition, which simplifies the data processing. Additionally, we introduce a modified low dispersion tube cell design. Compared to earlier “tube cell” designs, the new “parallel flow ablation cell” is less affected by sample surface to tube distance, maintaining signal duration of ˜5 ms when ablating NIST SRM 610 using 5 µm spot size.

All experiments were performed with a GeoLas C (193 nm excimer laser, Lambda Physik, Goettingen, Germany), the in-house build low-dispersion parallel flow ablation cell, and an ICP-TOFMS (icpTOF2R, TOFWERK, Thun, Switzerland). Hole drilling imaging was performed using 25 laser pulses on the same sample position with repetition rate of 100 Hz. Fast imaging was performed using single laser pulses per sample position with repetition rate of 100 Hz. Quantification for each pixel was carried out using NIST SRM 610 as external standard and with the 100% normalization of the sum of all metal oxides from Na to U [6].

Imaging using the hole drilling imaging mode of a garnet sample from a high-pressure rock was carried out with a speed of 2.5 sample positions per second. 25 laser pulses were fired on a sample position, which enhances pixel signal intensity, resulting in higher sensitivity, lower limits of detection, and element images with increased contrast without loss of lateral resolution. Fast imaging of a hedenbergite-ilvaite sample collected from the historical Fe-skarn ore deposit of Torre di Rio (Island of Elba, Italy) was performed to demonstrate the suitability of the parallel flow ablation cell for 100 Hz single pulse imaging of a real geological sample.

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