APPLICATION OF A 3D-PRINTED, HIGH EFFICIENCY SAMPLE INTRODUCTION SYSTEM FOR SINGLE CELL ICP-MS ANALYSIS

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Abstract
In this study, the application of an alternative, 3D-printed sample introduction system (SIS) is presented for single cell ICP-MS analysis. The SIS utilized in the experiments was designed by our group with the purpose to enhance single particle detection capabilities. It is able to provide higher transport efficiencies, increased sensitivity, lower background signals and a wider range of detectable particle size in single particle ICP-MS (spICP-MS) analysis compared to the standard SIS. Capitalizing on these features, two single cell ICP-MS (scICP-MS) experiments were done, using single cellular algae (Chlorella) and human endothelial cell suspensions, thereby demonstrating the SIS's ability to facilitate cell biology related studies such as determining the elemental composition/metal uptake of a single cell or monitoring its cell-to-cell variation in cell cultures.

Introduction
Single particle ICP-MS is a relatively novel analytical technique, which was first proposed by Degueldre et al [1], in around 2003. It is able to provide information about the elemental and isotopic composition, size distribution, particle number concentration etc. of nano- and – as later has been also shown – microparticles [2]. Since then, the method has been utilized for various purposes in material science and cell biology [3, 4]. However, the efficiency and applicability of the technique are severely hindered by the inefficient operation of the commonly used ICP-MS sample introduction systems (e.g. a concentric nebulizer coupled with a double-pass or cyclonic spray chamber). These systems are designed to produce as stable and fine aerosol as possible, ensuring plasma stability. However, this comes with undesired features from the perspective of single particle analysis such as low transport efficiency and narrow transmitted droplet size range [5]. To tackle this problem, sample introduction systems (SIS) designed to facilitate efficient particle detection started to appear recently. These systems vary in design, however they usually operate with nebulizers with decreased nozzle dimensions that require lower sample uptake rate, and are often complemented with additional (sheath) gas flows to promote higher droplet transmission [6, 7]. Utilizing these alternative SIS, increased particle detection efficiencies, decreased limit of detections (LODs), and lower sample consumption were reported. However, they are not part of the general ICP-MS laboratory equipment, partly because they pose a significant expense. Last year we presented the design

process [8] and later, the application [9] of the first completely 3D-printed version of such a high efficiency sample introduction system for ICP-MS. The system consists of a microconcentric nebulizer coupled with a single-pass spray chamber equipped with an optional sheath gas flow. In this study our goal was to assess the applicability of this system for cell biological purposes, and to compare the capabilities of the 3D-printed SIS to that of the standard one.

Experimental

An Agilent 7700X inductively coupled plasma mass spectrometer was used, coupled with its standard sample introduction system (MicroMist concentric nebulizer coupled with a "Scott-type" double-pass spray chamber) or our custom, 3D-printed sample introduction system (microconcentric pneumatic nebulizer coupled with a single-pass spray chamber equipped with optional sheath gas flow). During spICP-MS measurements, the data acquisition software was set to time resolved analysis (TRA) mode, and the integration time was set to 12 ms. In every experiment the plasma was operated with 15 $L \cdot min^{-1}$ of plasma gas flow rate, 1550 W of R.F. forward power and 10 mm sampling depth. The standard SIS was used with its regular 1.05 $L \cdot min^{-1}$ nebulizer gas flow and 750 $\mu L \cdot min^{-1}$ sample uptake rate with no sheath gas, whereas the 3D-printed SIS was operated with 1.35 $L \cdot min^{-1}$ nebulizer gas flow, 33.3 $\mu L \cdot min^{-1}$ sample uptake, and 0.1 $L \cdot min^{-1}$ sheath gas flow rate.

The high efficiency sample introduction system was printed with a ProJet MJP 3600 MAX type 3D-printer equipped with VisiJet M3 resin (3D Systems, Rock Hill, SC, USA).

In all experiments, trace-quality de-ionized water was used as a diluant. which was obtained from a MilliPore Elix 10 device equipped with a Synergy polishning unit (Merck, Darmstadt, Germany).

For experiments with algae cells, one strain of single cellular algae (d=3.6 μm) was used, which was the Chlorella sp. (MACC-360), obtained from the Monsonmagyaróvár Algae Culture Collection (MACC, Monsonmagyaróvár, Hungary). The cells were cultivated in the form of cell suspensions at 25°C with continous shaking, in an incubator which provided 16:8 h light-dark cycles. Tris acetate phosphate (TAP) medium was used as the growth medium for all culture, which prior to the spICP-MS measurements was replaced with de-ionited water through 5 repeated cycles of cell sedimentation via centrifuge and supernatant replacement. The extra nickel was added to the algae suspension using an analytical grade nickel standard solution (Merck, Darmstadt, Germany)

In experiments involving endothelial cells, human vascular endothelial cells (d=15.4) were used, grown in a dedicated endothelial cell culture medium (ECM) complemented with 5% of fetal bovine serum, 1% of endothelial growth supplement, and 0.5% of gentamincin, all obtained from ScienCell Research Laboratories (ScenCell, Carlsbad, CA, USA). After confluation, the cells were detached from the 60mm Petri dishes they were grown on using a trypsin-EDTA solution. The cells were washed with ECM and after centrifugation, resuspended in PBS (phosphate buffered saline), then fixed with 1% of paraformaldehyde.

Results and discussion

To demonstrate some of the benefits of our 3D-printed sample introduction system can provide, first an experiment was done on a series of cell cultures from the Chlorella algae strain. Four parallel cell cultures, with approximately $5 \cdot 10^6$ mL^{-1} cell number concentration, were prepared with different Ni^{2+} content in them, ranging from 0 (control) to 150 μM. The cultures' state was examined for 72 hours in every 24 hours via a series of spICP-MS measurements, monitoring the cell population, and cell-to-cell variation of the accumulated Ni^{2+} content. As Figure 1a shows, the added Ni^{2+} ions significantly affected the proliferation rate of the algae in the

concentration range used. The cell culture with 50 µM showed significantly higher growth rate as compared to the control sample. Maximum population growth factor could be observed on the culture with 100 µM of Ni^{2+}, although its proliferation was temporarily suppressed initially by the elevated heavy metal content, whereas for the 150 µM culture, population growth was suppressed throughout the whole experiment. Accumulated nickel content in individual cells above detection limit (LOD) was only observable in the case of the culture with the highest concentration. Figure 1b shows the trends related to the heavy metal uptake. Nickel levels appears to be constant for the first two days, then it starts to increase further, whereas LOD values were increasing gradually due to the increasing Ni background in the samples, which probably originate from the cells, that released them after medium changes.

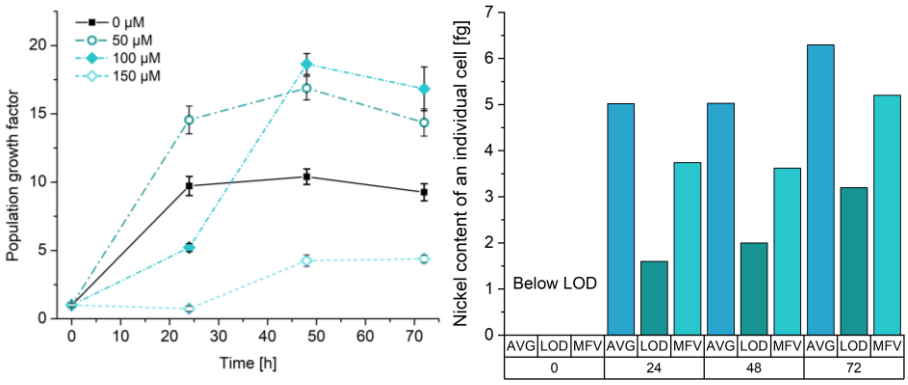

Figure 1. a) Population growth of Chlorella cell cultures in media with different concentrations of Ni^{2+} ions. b) The extent of the accumulation of Ni content in individual Chlorella cells, cultivated in a media with 150 µM Ni^{2+} of ions. The presented statistical indicators were calculated from the Ni content distribution of a few hundred cells (AVG: average, MFV: most frequent value, LOD: limit of detection)

In another experiment, we demonstrated the counting of human endothelial cells. The challenging part of carrying out such measurements comes from the relatively large size (d=15.4 µm) and fragility of these cells.

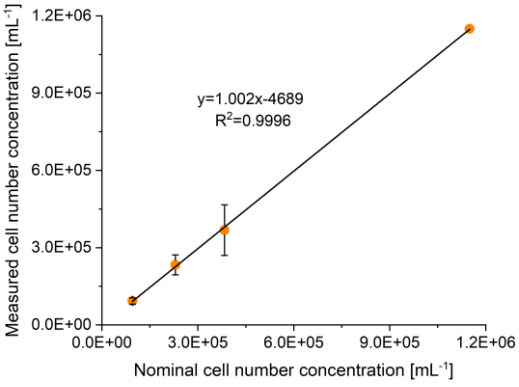

Figure 2. Correlation between the nominal and the determined cell number concentration of endothelial cell suspensions with different dilution rates. Cell detection was done based on the Ni content of the cells, measured at mass 58.

Optical particle counter experiments revealed that this size is above the cut-off size for the standard SIS, whereas the new system has transmission even for droplets with diameters as

large as 25 μm, thus making the detection of cells up to this size possible [9]. In the experiment, the endothelial cell dispersion with a cell number concentration of $1.15 \cdot 10^6$ mL^{-1} was diluted in multiple steps until a 12-fold dilution. The cell number concentration of each diluted sample was determined by scICP-MS measurements. The obtained concentation data shows a good linear correlation with the theoretical values (Figure 2), thus demonstrating that the counting of cells of this size is feasible with a decent precision using the new sample introduction system.

Conclusion

Both of the showcased cell biological experiments were made feasible by utilizing of the new, 3D-printed, plastic sample introduction system, as it has provided greatly improved transport efficiencies (the cell fraction that could be detected) compared to what can be achieved with the standard SIS. Furthermore, nickel content of individual cells was only above LOD when the 3D-printed system was used, due to its ability to enhance sensitivity and suppress background signals. As these results indicate, a perfectly functional ICP-MS sample introduction system could be made – for the first time in the literature – using only 3D-printing techniques, that has substantial benefits when applied in scICP-MS measurements.

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References

[1] C. Degueldre, P.Y. Favarger, Colloids Surf. A: Physicochem. Eng. 217 (2003) 137–142.
[2] H. Haraguchi, J. Anal. At. Spectrom. 19 (2004) 5–14.
[3] M. Corte-Rodríguez, R. Álvarez-Fernández, P. García-Cancela, M. Montes-Bayón, J. Bettmer, Trends Anal. Chem. 132 (2020) 116042.
[4] S. Miyashita, A.S. Groombridge, S. Fujii, A. Minoda, A. Takatsu, A. Hioki, K. Chiba, K. Inagaki, J. Anal. At. Spectrom. 29 (2014) 1598-1606.
[5] M. Tharaud, P. Louvat, M.F. Benetti, Anal. Bioanal. Chem. 413 (2021) 923-933.
[6] K. Inagaki, S. Fujii, A. Takatsu, K. Chiba, J. Anal. At. Spectrom. 26 (2011) 623-630
[7] F. Lin, S. Miyashita, K. Inagaki, Y.H. Liu, I.H. Hsu, J. Anal. At. Spectrom. 34 (2019) 401-406.
[8] Gy. Kajner, Á. Bélteki, M. Cseh, B. Vajda, Zs. Geretovszky, G. Galbács, Proceedings of 28th ISAEP, Szeged, Hungary, 2022.11.14.
[9] Gy. Kajner, Á. Bélteki, M. Cseh, B. Vajda, Zs. Geretovszky, E. Papp, M. Braun, G. Galbács, Proceedings of the EWCPS 2023, Ljubljana, Slovenia, 2023.01.29.