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Real matrix-matched calibration strategy for the quantification of neurodegeneration-related proteins in single human epithelial cells by LA-ICP-MS using specific metal-labelled antibodies

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Biological heterogeneity is a well-known fact that affects the study of biological processes, especially regarding cells. At present, there is a current need of developing new analytical methodologies allowing for the determination of elements and biomolecules in a cell-to-cell basis [1]. In this regard, laser ablation (LA) coupled to ICP-MS is a promising complementary alternative to liquid nebulization single cell (sc) ICP-MS for the characterization of individual cells. Furthermore, not only the analysis of elements naturally present in the cells can be tackled but also specific biomolecules through the combination of LA-ICP-MS with adequate metal-labelling strategies [2]. In this way, the qualitative 2D-mapping of target biomolecules with a subcellular resolution can be performed. However, the persistent lack of adequate matrix-matched reference materials still hinders the quantitative analysis of elements and biomolecules in biological samples by LA-ICP-MS, being especially critical in cell cultures due to their complex matrix.

In this work, we propose a novel matrix-matched calibration strategy, which fully mimics the matrix of cultured cells, by using the same cell line of the sample to create laboratory standards. As a case of study, the sequential quantification of two cytosolic proteins (MT2A and APOE) in individual human retinal pigment epithelial (HRPEsv) cells, both in cells subjected to inflammation with cytokine Interleukin-1α and control, was carried out. For such purpose, a single biomarker strategy using well-characterized Au nanoclusters (AuNCs) as specific antibody labels was performed for the proteins tagging. The laboratory standards were created by supplementing HRPEsv cells with suspensions containing nude AuNCs (HRPEsv@AuNCs cells). The preparation and characterisation of the single-cell laboratory standards (by both ICP-MS and LA-ICP-MS) were optimised as well as the data treatment protocol required for obtaining the quantitative distribution of the proteins in individual cells. To corroborate the quantitative results obtained for the proteins determination by LA-ICP-MS in HRPEsv cells, sc-ICP-MS analysis and commercial ELISA kits were employed.

[1] P.E. Oomen, M.A. Aref, I. Kaya, N.T.N. Phan, A.G. Ewing, Anal. Chem. 91 (2019), 588-621.
[2] A. Lores-Padín A, P. Menero-Valdés, B. Fernández, R. Pereiro. Anal. Chim. Acta. 1128 (2020) 251-268.

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