Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17103 (E-COST-GRANT-CA17103-4ec5c4de)

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Details of the STSM

Title: Simultaneous detection of proteins in individual cells by sc-ICP-ToF-MS

Start and end date: 21/09/2022 to 31/10/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

(max. 500 words)

The STSM took place at the 1.1 Division Inorganic Trace Analysis of the Bundesanstalt für Materialforschung und -prüfung (BAM) in Berlin (Germany), under the supervision of the head of Division Dr. Björn Meermann. The objective of the STSM was the development of a new analytical methodology for the simultaneous determination of specific biomolecules concentration in individual cells. For such purpose, the combination of the host group knowledge and expertise in the ICP-ToF-MS technique with the grantee´s knowhow in nanobiotechnology and cell culture handling has been ideal.

During the STSM different metal nanoclusters (MNCs: AuNCs, IrNCs, and PtNCs) were investigated for obtaining metal labelled immunoprobes. This probes, were employed in an immunoassay for labelling specific proteins related to neurodegenerative diseases. In particular, ferroportin (FPN), hepcidin (HP) and metallothionein 2 (MT2) proteins were studied in an *in vitro* cellular model of ARPE-19 cells (human immortalized cells from retinal pigment epithelium; RPE). Both control and cells subjected to two different oxidative stress treatments with glucose (100 mM, 48 h) or APPH (5 mM, 1 h) were studied. After the immunocytochemistry protocol of ARPE-19 cells with MNCs, FPN, HP and MT2 proteins were quantitatively determined in individual cells by single cell (sc) ICP-ToFMS.

The experimental work carried out in the STSM consisted in:

1. Optimization of the cell introduction into the ICP-ToF-MS. High transport efficiency is a crucial parameter in sc-ICP-MS analysis to ensure that a representative number of cells from the cell suspension

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

are introduced into the ICP-MS. The new microFAST sample introduction system (ESI) was coupled to the ICP-ToFMS for the analysis of ARPE-19 cells. Optimization of cells concentration in solution (in the range from $5 \cdot 10^3$ to $1 \cdot 10^6$ cells mL^{-1}), the sample volume (10-100 μL) as well as the ICP-ToFMS parameters (e.g., gas flows, lens voltages, torch position, attenuation of matrix elements, etc.) were carried out using control ARPE-19 cells.

2. Selection of the most adequate label (MNCs) to perform the simultaneous determination of three target proteins by sc-ICP-ToFMS in ARPE-19 cells. FPN, HP and MT2 proteins are present in ARPE-19 cells at very low concentration levels (in the order of ag-fg per cell) and, therefore, high sensitivity is required for their accurate and precise determination. Different analyses were carried out with control ARPE-19 cells by sc-ICP-ToFMS in order to select the most adequate label for each protein of interest. Experimental results showed that the high amplification factor provided by MNCs allowed to determine the three proteins in ARPE-19 cells: AuNCs (amplification factor = 220 Au atoms per antibody molecule) were used as the elemental label for FPN, IrNCs (amplification factor = 1760 Ir atoms) for HP, and PtNCs (amplification factor = 1200) for MT2.

3. Optimization of the immunocytochemistry (ICC) assay for labelling the proteins of interest. In order to preserve the cells integrity during sc-ICP-ToFMS analysis, two different fixation protocols were evaluated with ARPE-19 cells. Finally, cellular fixation was performed with 4% (v/v) PFA and incubated 10 min at room temperature. Additionally, ICC was optimized in terms of the antibody concentration for each immunoprobe (AuNCs:FPN, IrNCs:HP, and PtNCs:MT2).

4. Quantification of specific proteins in control and treated ARPE-19 cells by sc-ICP-ToF-MS. FPN, HP and MT2 concentrations were determined by sc-ICP-ToFMS in control and treated (glucose and APPH) ARPE-19 cells. Stress conditions seem to induce an antagonist effect on ARPE-19 cells, showing up- or down-regulation of the proteins *versus* control conditions.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

(max. 500 words)

During the STSM, the grantee received training in ICP-ToF-MS for single cell analysis. This technique was successfully employed for the simultaneous determination of FPN, HP and MT2 proteins in an oxidative stress cellular model. As the methodology has been already developed, it could be further employed in multiple applications. For example, it could be applied to study the internalization of antisense oligonucleotides (ASO) in individual cells and elucidate the interaction of ASOs with different cellular models and treatments.

The antisense oligonucleotides labelling could be done by bioconjugation to MNCs, as a strategy to introduce a metal tag detectable by mass spectrometry (MS) techniques. MNCs contain hundreds of detectable atoms by MS, amplifying the signal of the target analyte and making affordable its detection at low concentration levels. Moreover, if compared to other traditionally employed metal tags (such as quantum dots, nanoparticles or lanthanide polymers), MNCs suppose a lower risk of hindering the biological functions of the ASOs due to their relative small size (metal core < 3nm). As has been observed for FPN, HP and MT2 proteins in the *in vitro* cellular model of ARPE-19 cells, it would be necessary to optimize the MNCs label (taking into account its amplification factor) depending on the target analyte: analytes at low concentration levels require elemental labels that provide high amplification for MS detection.

Additionally, this labelling strategy could be also employed for bioimaging of the antisense ASOs in different biological models (e.g., cells and tissue sections) by using a laser ablation system coupled to ICP-MS (spatial resolution at subcellular level can be obtained). This could be of special interest to

determine the spatial distribution of the antisense oligonucleotides within cellular and organ structures that have been subjected to ASO therapy.

The results of this STSM will be presented as an oral contribution in the international conference “European Winter Conference on Plasma Spectrochemistry” that will take place in Ljubljana (Slovenia) from the 29th of January to the 3rd of February 2023. Moreover, obtained results would lead to the publication of a scientific paper. The article will be a collaboration between the University of Oviedo (Spain) at BAM in Belin (Germany), strength the multidisciplinary work among different research groups in Europe.