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

Action number: CA20129

Grantee name: Sara Alexandra Carvalho Lopes de Freitas

Details of the STSM

Title: Synergistic effect between photothermal and radiotherapy using plasmonic nanoparticles as photo-absorbing agents and radiosensitizers toward higher-efficiency colorectal cancer treatments

Start and end date: 25/02/2025 to 31/03/2025

Description of the work carried out during the STSM

During this STSM, I conducted a systematic investigation of the effects of proton therapy and gold nanorods (AuNRs) on colorectal cancer cells, aiming to explore their potential as radiosensitizers and photothermal agents. The research involved studies on cellular viability and ROS (Reactive Oxygen Species) production following proton irradiation and photothermal therapy (PTT) individually, as well as nanoparticle (NP) internalization studies.

Proton Therapy Studies:

- Experiments were conducted on colorectal cancer cells (HCT116) with different radiation doses (2, 5, 7.5, 10, and 15 Gy).
- Cell viability and ROS production were assessed at various time points (24, 48, 72, and 96 hours post-irradiation).
- A second set of experiments incorporated gold nanorods functionalized with folic acid (incubation time: 3h30) to evaluate their radiosensitizing effects. No significant differences in cell viability were observed, suggesting either unsuccessful functionalization or insufficient internalization time.
- This study was performed twice (N=2), at two scheduled beam times: March 11 and March 24.

Nanoparticle Internalization Studies:

- Given the lack of significant results from the functionalized AuNRs, an internalization study was performed. The internalization of both functionalized and non-functionalized AuNRs was assessed at concentrations of 25, 75, and 100 μg/mL over different incubation periods (2, 4, 6, and 24 hours).
- This study aims to determine the amount of gold effectively penetrating the cells.

[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.

Photothermal Therapy (PTT) Studies:

- A previously developed photothermal therapy setup was mounted, and tests were conducted using AuNRs. Samples were irradiated (5 minutes) with a continuous wave laser (808 nm) using two different nanoparticle concentrations (25 and 100 μg/mL). Four laser fluences were tested, ranging from 0.6 to 3 W/cm².

Cell viability assessment:

- Following photothermal and proton irradiation, the cell viability was assessed through clonogenic and Alarmar Blue viability studies.

Overall, my time at Universidad Autónoma in Madrid was dedicated to advancing our understanding of the interactions between gold nanorods and biological systems, as well as exploring innovative approaches to cancer therapy using proton irradiation.

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

This STSM successfully addressed several key objectives outlined in the original proposal. While some experiments provided clear and promising results, others revealed challenges that require further investigation. The work conducted has deepened our understanding of the interactions between proton therapy, nanoparticles, and colorectal cancer cells, laying the groundwork for future refinements and collaborative research.

Main results/conclusions:

- Dose-Dependent Response in Proton Therapy: A clear trend in cell viability reduction was observed with increasing proton doses over time. At 96 hours post-irradiation, cell viability was observed to be below 20% for radiation doses above 7 Gy.
- Nanoparticle Internalization Data: The study on NP internalization will provide crucial information on the efficiency of AuNRs uptake by cells and its potential impact on therapy.
- Photothermal Therapy Testing: Initial results demonstrated a time-dependent photothermal effect with different laser fluences and AuNRs concentrations.

Planned Follow-Up Activities:

- **Further Internalization Studies:** Future experiments will focus on optimizing NP functionalization and internalization conditions to improve efficacy. Tests involving the utilization of a PEG- SH to facilitate the conjugation of the folic acid on the gold surface are already in development.

- **Combination Therapy Refinement (Protons + AuNRs):** Adjusting incubation times and testing alternative functionalization strategies to enhance the radiosensitizing effects of AuNRs.

- **Extended Photothermal Analysis:** Additional studies will refine PTT parameters for maximum efficiency. This includes testing different irradiation times, as well as more fluences in the 1 – 3 W/cm2 range, which was the interval where a more abrupt decrease in cell viability was observed (for the 100 μg/mL concentration).

Also, as mentioned in the Application Form, I believe that the proposed experiments align with the objectives outlined by the Action Cost, particularly within the domains of radiosensitising properties of metal-based nanoparticles exposed to radiation and the development of novel treatment protocols, predicted in the main objectives of MultIChem COST Action. In particular, to the WG3 regarding the inclusion of the radiation-driven nanoscale effects into the existing treatment plans based on macroscale dose delivery to facilitate technological advances (Tasks 1 and 3).