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

Action number: CA20126

Grantee name: Francisco Carvalho

Details of the STSM

Title: Magnetic nanostructures grown in nanoporous alumina templates for biotechnological applications

Start and end date: 28/05/2023 to 07/06/2023

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 main goal of this STSM was to study the effect of template-assisted electrodeposited Fe NWs, fabricated in porous anodic alumina (PAA) membranes, on glioblastoma multiforme cell lines (U87) and to further test the nanostructures in vivo in collaboration with the General Hospital of Gregorio Maranon.

In this context, the high aspect-ratio Fe NWs, having a diameter of 50 nm and a length of 500 nm, were incubated overnight in U87 glioblastoma cells with different concentration, in order to study their biocompatibility and internalization times. To evaluate cell viability, the alamar blue assay was performed at three different time points: 24 hours, 48 hours, and 72 hours after the injection of NWs. The results obtained from this study provided insights into the effects of the NWs on cell viability and internalization dynamics, concluding that the NWs were successfully internalized 24 h post injection.

To assess the effectiveness of the Fe NWs for magnetic hyperthermia, cytotoxic effects were evaluated by subjecting the NWs incubated with U87 glioblastoma cells to an alternating magnetic field (AMF) for 30 minutes. The AMF was generated using a magnetic field of 150 Oe, and a generator operating at two distinct frequencies: 45 kHz and 180 kHz. Subsequently, the cell viability was evaluated via the alamar blue assay at two different time stamps, namely 24h and 72h, after the exposure to the AFM. This assessment provided information on the cytotoxic effects of the NWs under the influence of the applied magnetic field and allowed us to conclude that a decrease of cell viability is observed at both

[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.

frequencies.

The magnetic hyperthermia tests were performed at the Faculty of Medicine of UAM, while the cell culture and viability tests were carried out at the Applied Physics Department of the Faculty of sciences in the UAM campus.

In addition, the STSM also included the fabrication of Magnetite Nanoparticles (NPs) using a hydrothermal method. The process involved a mixed water-based solution of two compounds: Iron(III) chloride and Ammonium dihydrogen phosphate. This solution underwent a thermal treatment at 220ºC, resulting in the formation of hematite NPs. The ratio of the two compounds determined the shapes and sizes of the NPs, which were produced at the nanometer scale. In this framework, nano-needles and nano-ellipses were successfully synthetized. To convert the hematite NPs into magnetite NPs, further thermal treatment was carried out in a reductive environment. This step transformed the hematite NPs into the desired iron oxide (magnetite) NPs. These fabrication processes took place at the Applied Physics Department of UAM.

Furthermore, on May 6, in vivo experiments were conducted in collaboration with Dr. Mª Victoria Gomez-Gaviro at Hospital General Universitario Gregorio Marañón. These experiments involved the use of mice as animal models to assess the biodistribution of the different types of fabricated NPs within the brain. The goal was to understand how the NPs under study were distributed in the brain tissue of the mice under the application of an external magnetic field, providing valuable insights into their potential applications for brain-related diseases.

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)

The STSM has successfully achieved its major goal of using magnetic nanostructures, specifically the iron NWs grown in nonporous alumina templates, for biotechnological applications. The research conducted in this STSM has contributed to the investigation of current and innovative clinical needs, within the framework of the COST Action. Particularly, no significant reduction of cell viability was observed for the cells internalized with NWs that were not subjected to the external magnetic field. From this study, it could be concluded that, at the tested concentrations, the Iron NWs are biocompatible and fully internalized after 24h.

To assess the effectiveness of the Fe NWs for magnetic hyperthermia, cytotoxic effects were evaluated by subjecting the NWs incubated with U87 glioblastoma cells to an alternating magnetic field (AMF) for 30 minutes. In this context, a significant reduction of cell viability occurred 24h after the magnetic field exposure. Moreover, 48h after exposing the cells to the AMF, a further reduction of cell viability was observed for the treated cells. No significant difference in the viability assays was observed when changing the frequency of the AFM from 45kHz to 180kHz.

These experiments will continue to be performed to test NWs with different lengths and diameter, to compare the data and subsequently, publish the obtained results.

Furthermore, we performed in vivo experiments to observe the biodistribution of the NPs, injected at different concentrations, (Nanowires, nano-needles, and nano-ellipses) within the brain, at different time stamps. From these studies, it could be concluded that, regarding the NWs, a higher concentration of NPs is needed, while for the magnetite NPs, the concentration of NPs still needs to be optimized, to observe the accumulation of the particles within the brain. In these context, future in vivo experiments with nanowires and magnetite NPs will be planned, aiming at studying their biodistribution, the induction of cancer cell death through magneto-mechanical actuation, magnetic hyperthermia, and their suitability as magnetic resonance imaging contrast agents, allowing the evaluation of their potential for theragnosis.

Additionally, magneto-mechanical cell death experiments with different fields and frequencies will also be performed, to evaluate the ability of the NWs to kill cancer cells through a magneto-mechanical action instead of using the increase of temperature as employed in magnetic Hyperthermia.