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

Action number: CA20126

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Details of the STSM

Title: Biological characterization of chitosan-based scaffolds for simultaneous cancer treatment and bone tissue regeneration

Start and end date: 01/09/2022 to 31/10/2022

Description of the work carried out during the STSM

Overall, during these two months, the biological characterization of the materials was successfully accomplished. The first 2 weeks were mainly dedicated to the training that are required to work in the laboratories which included the reading of tutorials and a final evaluation through a theoretical test.

In the beginning of the laboratorial work, we faced with a difficulty since the scaffolds floated on culture medium making the cell seeding unfeasible. Thus, the first step was to study a way to fixate the scaffolds into the bottom of the culture plates. Two methods were evaluated: fixation employing a polydimethylsiloxane solution (PDMS) or applying a centrifugation and both approaches showed to be effective.

Regarding the cell lines we pretended to use Human Mesenchymal Stem Cells (hMSCs) but this cell line grows very slowly. Therefore, we change the cell line to human osteoblast-like osteosarcoma (Saos-2) since they are bone cancer cells, grows faster and is regularly used in bone cancer study as a model for presenting of new treatments. Even though, with hMSCs we were still able to access the cell attachment and morphology for 7 days of culture for the following samples: Chitosan-2.5% w/v, Chitosan-3.5%-nHA-60/40, Chitosan-3.5%-Fe_3O_4-10%, Chitosan-3.5%-Fe_3O_4-20%, Chitosan-3.5%-Fe_3O_4-10%-nHA-60/40 and Chitosan-3.5%-Fe_3O_4-20%-nHA-60/40, where nHA-60/40 is nano-Hydroxyapatite that was incorporated at a 60:40 chitosan n-HA ratio. 10 and 20% w/w respects to incorporated Fe_3O_4 nanoparticles.

For the Saos-2 cell line we performed the following assays:

[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.

(1) Swelling assay with phosphate-buffered saline (PBS) and culture medium was added to the proposal assays. All the samples were analysed after 1,4 and 7 days.

(2) Cell viability: after 3, 7 and 14 days the seeded scaffolds were stained with Calcein-AM and propidium iodide (PI) followed by observation under a confocal microscope. The samples Chitosan-2.5% w/v, Chitosan-3.5%-nHA-60/40, Chitosan-3.5%-Fe_3O_4-10%-nHA-60/40 and Chitosan-3.5%-Fe_3O_4-20%-nHA-60/40 were studied.

(3) Cell morphology and attachment: after 3, 7 and 14 days the seeded scaffolds were stained with phalloidin and 4′,6-diamidino-2-phenylindole. All the samples were afterwards observed using the confocal microscope.

(4) Mineralization: the initial proposal was to perform Alizarin red staining although, the dye binded into chitosan turning this assay impractical. Thus, scanning electron microscope (SEM) analysis with energy-dispersive X-ray (EDX) was selected to evaluate the cells mineralization in osteogenic medium. All the samples were used to seed the cells, and the microscopy analysis are still needed to be carried out for 7, 14 and 21 days of culture.

(5) Assessment of osteogenic differentiation: due to the time limitation it was no possible to perform the qRT-PCR. In turn, the immunostaining for Osteopontin (OPN) and Alkaline phosphatase (ALP) was carried out for 7, 14 and 21 days of culture. The samples were observed under a confocal microscope

The cell metabolic activity assay was not performed due to the reduced available time and since an indirect MTT assay has been previously carried out.

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

The biological characterization of the produced scaffolds was fundamental to understand their potential for bone tissue engineering. The obtained results showed that our materials have great potential since they are biocompatible, the cells are attaching and proliferating not only in the scaffolds surface but also in inner zones. Preliminary results shows that the cells seeded on the scaffolds in special the ones containing magnetic nanoparticles are expressing Alkaline phosphatase (ALP) which is early marker of osteogenic differentiation. Complementing these results with the previous physico-chemical and magnetic analysis, we expect a scientific publication in the following next months.

This STSM allowed me to acquire knowledge and training in different *in vitro* assays that are vital for bone tissue engineering studies. I could experience distinct methods of working and organization that are very valuable. I had the opportunity to interact and exchange knowledge with people that works in different biomedical applications which increased my scientific knowledge on other fields.

In general, this collaboration was very fruitful for both home and host institution, and it is expected future cooperation and exchange.