STSM Activities Report

Arkaitz Mucientes Ruiz
Instituto de Investigación Sanitaria San Carlos (IdISSC)
arkaitz.mucientes@salud.madrid.org

Supervisor: Prof. Benjamín Fernández-Gutiérrez
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STSM title: *"Study of the differential behaviour in osteogenic differentiation of h-MSCs on nanoß-TCP and nanoHA scaffolds due to their origin"*
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Host: Prof. Fernando Jorge Monteiro
i3S, INEB, Universidade do Porto
fjmont@fe.up.pt

I applied for the STSM as an opportunity to expand the original draft of my PhD thesis and to increase my technical skills and knowledges in new methodologies, currently unavailable in my research center. Thus, the purpose of the stay has been to learn the synthesis, sintering and molding processes of bioceramic materials for further use as scaffolds in regenerative therapies.

For this purpose, we designed a work-plan, including several tasks. The final aim was to perform a comparative evaluation of the osteogenic capacity of different human mesenchymal stem cells (hMSCs) on nano beta tricalcium phosphate (nanoß-TCP) and nanohidroxyapatite (nanoHA). MSCs from three different localizations were used: adipose tissue (ASC), dental pulp (DP-MSC) and bone marrow (BM-MSC).

Task 1: Synthesis, sintering and sterilization nanoß-TCP and nanoHA scaffolds

The main goal was to prepare scaffolds of both compounds, enough for future experiments. During my stay I learned and performed the necessary protocols to obtain the scaffolds. Table 1 resumes the main features of these scaffolds.

Commercial nanoß-TCP and nanoHA, provided as powder, were kindly supplied by Fluidinova S.A. (Maia, Portugal). Samples of both nano ß-TCP and nano hidroxyapatite were molded as discs of 10 mm diameter prepared from 150 mg of dry prowder. A uniaxial compression stress of 80 bar was applied by a manual press (Metra Snow P3).

The sintering of the samples required different time and temperatures for each compound: 1150ºC with a *plateu* of 60 minutes for nanoß-TCP and 830ºC with a 15 minutes *plateu* for nanoHA.

Finally, the samples were sterilized by two methods:

1 By serial washing in 70% ethanol followed by saline serum and miliQ water.
2 By heating at 1500 ºC during 2 hours.

Table 1: Main features of the scaffolds obtained during the STSM

Compound	Amount (mg)	Diameter (cm)	Sintering temperature (ºC)	Sintering time (minutes)	Number of samples
Nanoß-TCP	150	10	1150	60	182
NanoHA	150	10	830	15	82

Due to technical problems, taking place during this task, the timeline of the rest of the designed work-plan was delayed. This task was considered successfully completed since sufficient number of scaffolds needed for the experiments were obtained.

Task 2: Thawing, cell culture and expansion

The main goal of this task was to obtain a sufficient number of cells to be seeded on the scaffolds from task 1.

Cells used in these experiments, belonged from my institution, IdISSC. Human mesenchymal stem cells (hMSC) from the three different origins – adipose tissue (ASC), dental pulp (DP-MSC) and bone marrow (BM-MSC) – have been used in order to investigate their potential behavioural differences. In total, 9 hMSCs samples were used, 3 from each origin.

Once they were thawed and counted, cell viability was tested using trypan blue before to expand them 175cm^2 in plastic flasks. Growth medium (DMEM, supplemented with 10% FBS and antibiotics; DP cells were supplemented with 20% FBS instead of the 10% usually established). Cells cultures were expanded at 37ºC in a 5% CO_2 atmosphere. Medium was changed every 3 days until they were seeded on the samples.

Task 3: Seeding the cells on the scaffolds

An experiment lasting 10 days was designed to evaluate the cell viability in a bioceramic scaffold context. Evaluation was performed at days 1, 3, 7 and 10 of culture, both under osteogenic stimulation and non stimulating conditions. Two different mediums were used: growth medium (DMEM, supplemented with 10% FBS and antibiotics; DP cells were supplemented with 20% FBS) and osteoinductive medium (StemMACS OsteoDiff Medium, Miltenyi Biotec).

Before cell seeding, the scaffolds were soaked in growth medium during 24 hours to ensure the cell adhesion to the bioceramics. According to Lobo *et al*, in order to enhance the osteogenic differentiation, a suspension containing 24.000 cells was seeded on each scaffold. Experiments were performed in triplicate.

Cell proliferation and viability was measured using the Alamar Blue™ indicator, following the manufacturer instructions. Briefly, the metabolic conversion by living cells of the non-toxic and cell permeable resazurin into resorufin gave a color change that can be monitorized measuring the absorbance at 570nm after 1-4 hours at 37ºC. The amount of absorbance was directly proportional to the number of living cells and corresponded to the metabolically active cells. At the established times the variation of absorbance was read using a spectrophotometer (Sunrise, TECAN).

Initial results (Figure 1 and Figure 2) indicate that both viability and cell proliferation showed higher values in nanoß-TCP samples, for any combination of hMSC origin and medium. These data are currently being analysed in order to obtain more exhaustive conclusions.

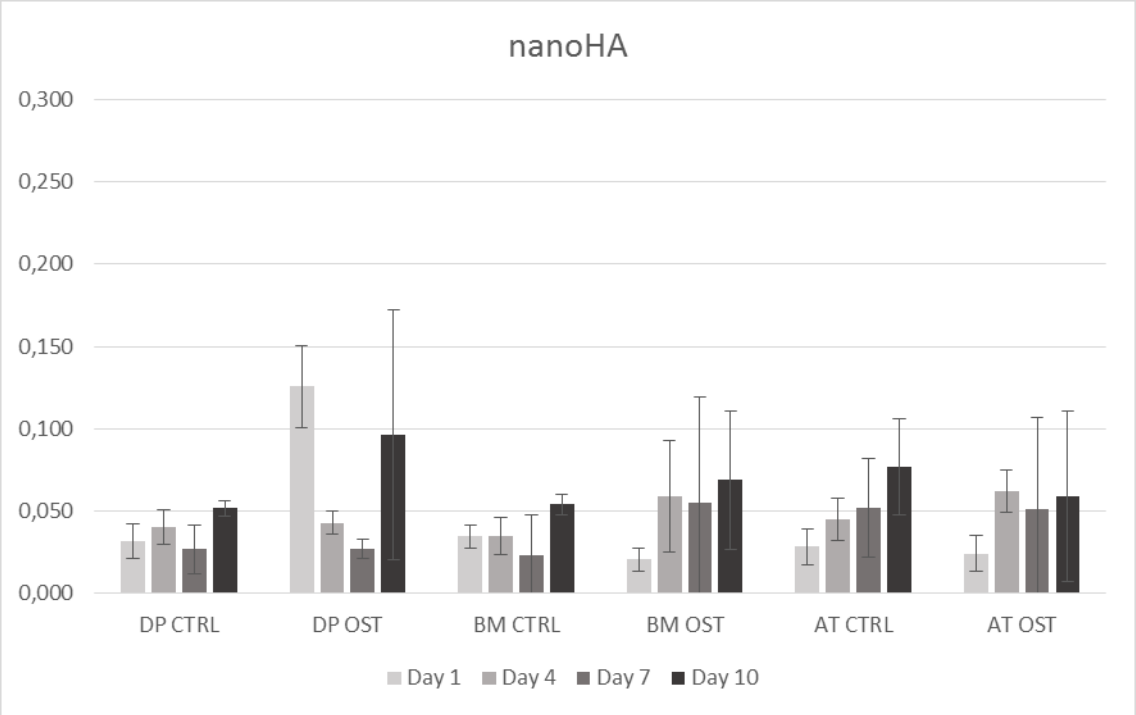

Figure 1: Alamar Blue results after in vitro seeding of 24.000 cells on nanoHA scaffolds cultures with growth (CTRL) and osteoinductive (OST) medium over a 10-day period.

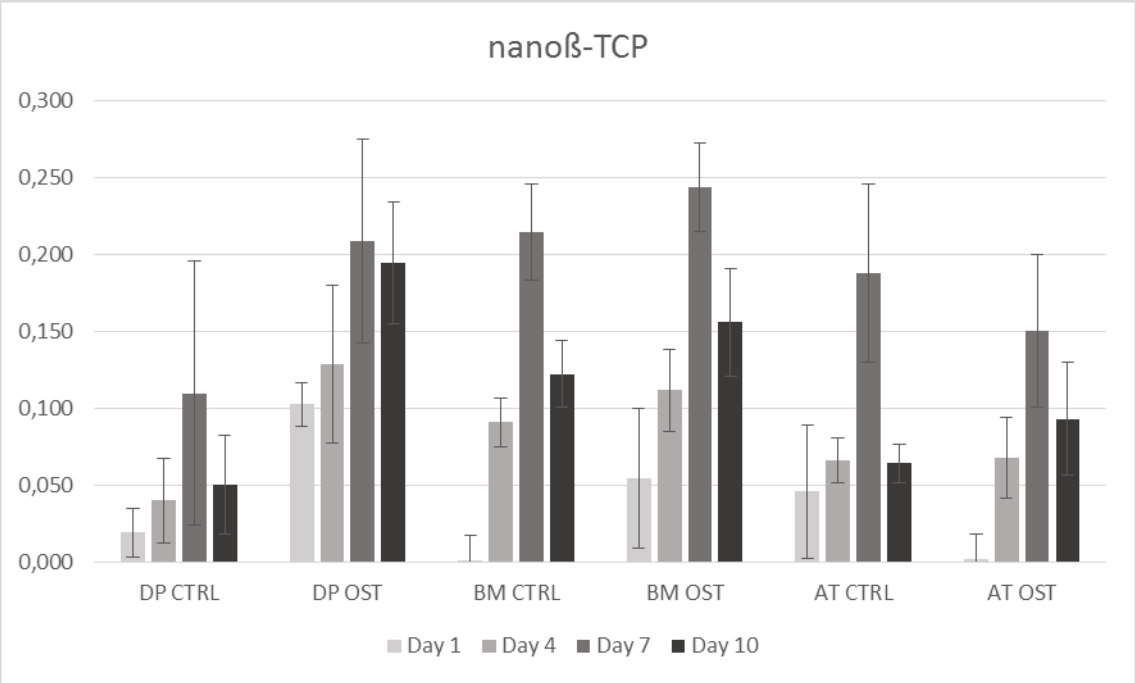

Figure 2: Alamar Blue results after in vitro seeding of 24.000 cells on nanoß-TCP scaffolds cultures with growth (CTRL) and osteoinductive (OST) medium over a 10-day period.

Task 4: Quantitation of early osteogenic expression of lineage specific genes.

As stated in Task 1 section, it was not possible to fully complete the task 4 before the end of my stay. However, during my stay I improved my skills to recover the cells from the biomaterials and to extract the genetic material necessary to perform the qPCRs. I used the RiboPure™ Kit (Thermo Fischer Scientific) to obtain purified RNA. RNA was quantified, frozen and stored at -20ºC before being sent to my home institution to complete the established task.

The necessary qPCRS for the total quantitation of early osteogenesis of cells cultured in bioceramic scaffolds will be done in my home institution. It will be done by measuring the expression of five genes: two house-keeping ($\beta_2 m$ and *PPiA*) and three osteogenic lineage specific genes (*RUNX2*, *COL I* and *ALP2*).

BIBLIOGRAPHY

Sonja E. Lobo, Robert Glickman, Wagner N. da Silva, Treena L. Arinzeh, and Irina Kerkis, "Response of stem cells from different origins to biphasic calcium phosphate bioceramics," Cell and Tissue Research, 2015.