SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: **CA17103- (DARTER)**
STSM title: **Precision nanosystems as nucleic acids delivery platforms**
STSM start and end date: **2020-02-20 - 2020-03-31**
Grantee name: **Lorena García Hevia**

PURPOSE OF THE STSM:

I want to use my knowhow in nanobiotechnology to engineer precision-targeted nano-vehicles to carry nucleic acids, for the local treatment of several tumours that also serve for detection/diagnosis.
The first objective of this ambitious project is the design, synthesis and characterization of several nanosystems carrying nucleic acids. Therefore, I would like to take advantage of the expertise of the Advanced (magnetic) Theranostic Nanostructures Lab in the Host Institution, international Nanotechnology Laboratory.
In summary, the main goals of these STSM were:

- Design, synthesis, and fabrication of several nanomaterials that will be used in the project, that is, silica spheres, magnetite nanoparticles, and even more complex nanostructures such as those with a core-shell morphology.
- Include fluorescent dyes or drugs into the nanoparticles
- Study drug release as a proof of concept experiment of tracking and delivery of different compounds.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

As mentioned in the STSM proposal, the experiments carried out were the following:

- **Synthesis of magnetite nanoparticles:** For the synthesis of dispersible magnetite (Fe_3O_4) nanoparticles (NPs) in organic medium, a necessary requirement for subsequent silica coating, a hydrothermal synthesis method is required. Thus, 8 mmol (1.59 g) of $FeCl_2 \cdot 4H_2O$ and 14 mmol (3.78 g) of $FeCl_3 \cdot 6H_2O$ were dissolved in 7 ml of water, which was added to 40 ml polytetrafluoroethylene beaker for autoclave. In adittion, 1.64 mmol (0.499 g) of sodium oleate (82%) was dissolved in 5 ml of water at 60 ° C. Once completely dissolved, this solution were added to the previous one of Fe, and then 15 ml of NH_4OH. After closing the beaker, went to the autoclave and sealing it, the reaction was left at 200 ° C for 24 hours.

The obtained product was recovered by decantation, first washed with water. After that, the NPs were allowed to dry 24 h in vacuum at room temperature. It was then resuspended in cyclohexane, and centrifuged for 10 min at 3000 rpm to remove insoluble solids. To wash off excess oleate, ethanol were added and everything was centrifuged for 10 min at 6000 rpm. Finally the NPs were resuspended in cyclohexane for preservation.

- Synthesis of Fe_3O_4 NPs coated with silica

Cyclohexane (8.73 ml), $Fe3O_4$ NPs in cyclohexane (270 µl of 7 mg/ml solution), NH_4OH (70 µl) and finally TEOS (150 µl) were mixed in the vortex and ultrasound bath for two minutes. After that, the vials were in the

dark for 20h. After this time, 6 ml of ethanol were added to the vial and it was shaken. To wash, it was first centrifuged at 3000 rpm for 5 min, resuspended in ethanol with sonication, and centrifuged again (10 min at 8000 rpm). This process was repeated 3 times. Finally preserve the NPs in ethanol.

- Synthesis of silica NPs: silica spheres were prepared using a modified Stöber method. Tetraethyl orthosilicate (1.7 mL, 98%) was added to a solution containing ammonium hydroxide (1.97 mL), water (3.1 mL), and ethanol (18.2 mL), stirring at room temperature for 2 h. The excess of reagents was removed by three centrifugation/redispersion cycles with ethanol (9,000 rpm, 10 min). Particles were finally redispersed and stored in ethanol.

- Silica nanoparticles loaded with DOX: Silica particles were functionalized with (3-aminopropyl)triethoxysilane (APS) by means of the addition of 0.25 mL of APS in 5 mL of an ethanolic dispersion of SiO2 (8.7 mg/mL). After a 3-h stirring, the excess of reagents was removed by three repeated centrifugation/redispersion cycles with ethanol (7,000 rpm, 20 min). Then, the APS-functionalized SiO2 particles were diluted in 10 mL of EtOH and added to 10 mL of an Doxorubicin solution. After a 3-h stirring, the excess of drug was removed by three repeated centrifugation/redispersion cycles (7,000 rpm, 20 min), affording an aqueous solution of doxorubicin silica particles.

- Synthesis and functionalization of the Mesoporous Silica-coated Magnetite (*)

- Characterization of nanosystems

- Hydrodynamic diameters and ζ-potential values of the nanoparticles were determined using the dynamic light scattering (DLS) measurements (Horiba Scientific SZ-100 instrument).
- The nanoparticle morphology was studied by transmission electron microscopy (TEM) (JEOL JEM-2100)
- The homogeneity of the nanoparticles was also confirmed by scanning electron microscopy (SEM) (FEI Quanta 650 FEG)

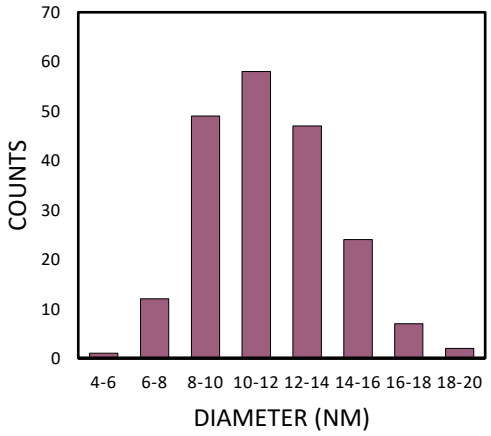
- Drug release studies

- The release profile of doxorubicin from silica nanoparticles was studied for 8 h in milli Q water at 25 ºC. It was monitored against time through high performance liquid chromatography (HPLC) using anisocratic gradient of water:acetonitrile (from 100% to 25:75%) and an Aeris 1.7 µm peptide XB-C18 column.

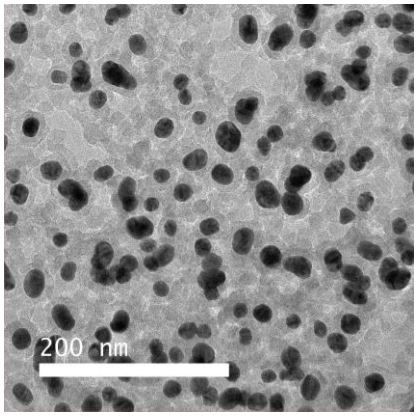
(*) Due to the Covid-19 pandemicI didn't have time to accomplish this experiment.

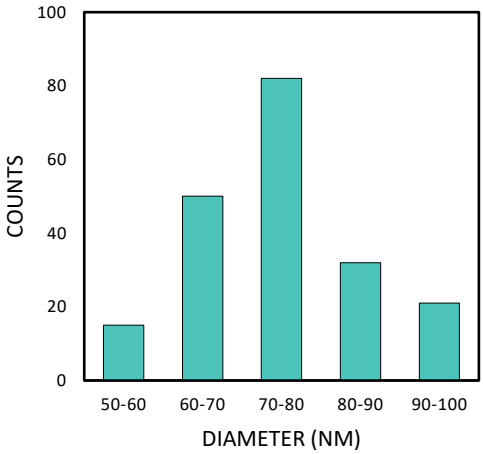
DESCRIPTION OF THE MAIN RESULTS OBTAINED

- Magnetite nanoparticles: size distribution of Fe_3O_4 NPs synthesized.

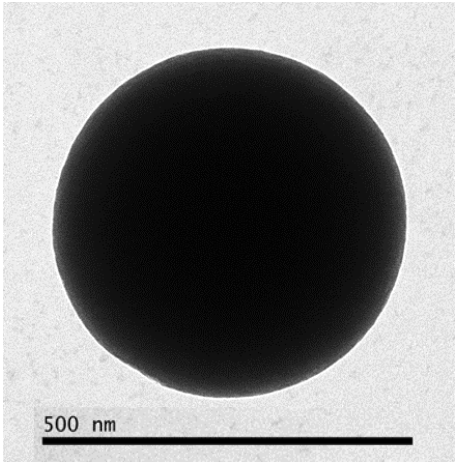


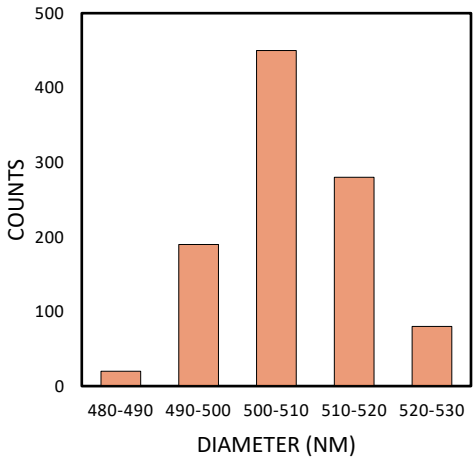
- Magnetite nanoparticles coated with silica: TEM and size distribution.

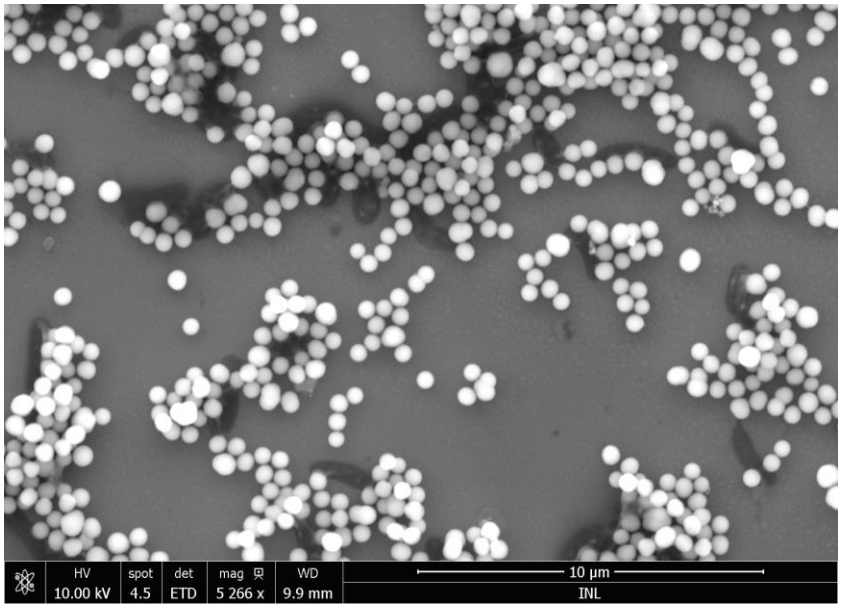




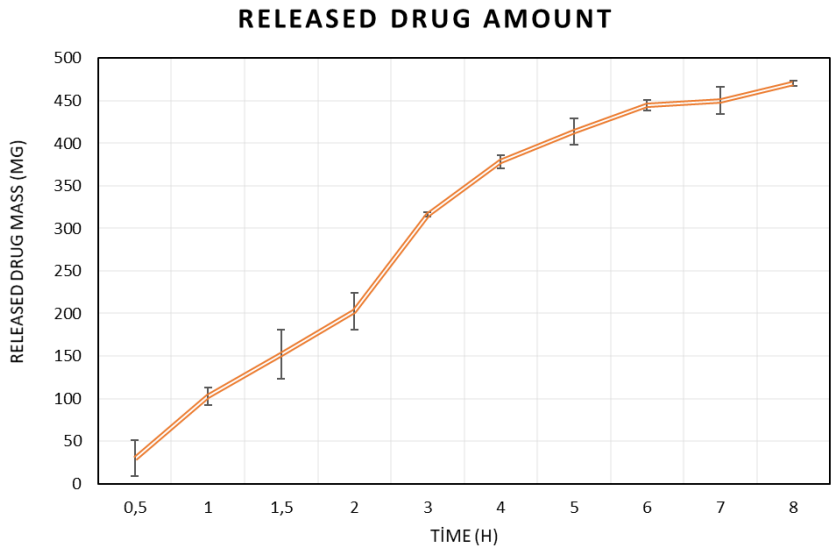
- Silica nanoparticles: TEM, SEM and size distribution.







- Drug release studies: Drug release profile from silica nanoparticles in milli Q water pH 5.5 at 25 ºC, after three replicas.



FUTURE COLLABORATIONS

This STSM has strengthened the collaboration between home institution (Nanomedicine group from Valdecilla research Institute IDIVAL) and host instituton (Amthena Lab from INL).
As I mentioned in the application, the final project is developing precision-targeted nano-vehicles to carry nucleic acids for the local treatment of several tumours that also serve for detection/diagnosis. In this sense, thanks to this STSM, it was possible to accomplish almost all of the first objective: synthesis and characterization of several nanosystems carrying nucleic acids. Unfortunately, due to the **Covid-19 outbreak**, I wasn't able to finish the functionalization goal which had been proposed in the application. I hope to have the opportunity to return INL in order to complete this goal.
In any case, I have satisfactory results to be able to continue carrying out this project as soon as return life to normality in Spain.
Finally, we have in mind the possibility of future staff visits, e.g. within Spanish-Portuguese projects, Cost Actions, Erasmus+ Programme, etc.