PROJECT ACRONYM

CUPIDO

PROJECT TITLE

Cardio Ultraefficient nanoParticles for Inhalation of Drug prOducts

Deliverable 2.3

EMD-mediated guidance of ^{Fe}CaP

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Table of Revisions

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Table of Contents

Index of Figures and Tables

1. Executive summary

The current deliverable 2.3 outlines a methodology and the relevant device to maximize the time of exposure of nanoparticles to cardiac cells employing an electromagnetic-mediated guidance of superparamagnetic iron doped CaPs (^{Fe}CaPs) (synthesized and characterized in WP1) to the heart via an electro-magnetic device. This strategy is alternative (or even complementary) to the chemical approach employing myocardial-specific internalizing aptamers (see D2.1) that can overcome any potential limitation due to the lack of a cell-specific receptor at the heart level.

This document provides part of the results extracted from the whole activities performed. Results of the theoretical simulations generated in D4.1 and related to the interaction between magnetic forces and fluidic forces have been experimentally confirmed, by using a static magnet featuring the required properties. In addition, a low pulsed electromagnetic bioreactor device has been developed and tested to control drug release efficacy from ^{Fe}CaPs. Micro-fluidic devices have been realized by 3D printer as an *in vitro* platform to mimic different physiological blood velocities. The stability of ^{Fe}CaPs has been assessed in these devices over time, when subjected to different physiological velocities and for different time periods.

Key deliverable achievements:

1. Selection of a magnetic stimulation system
2. Identification and experimental validation of magnetic parameters required to accumulate the FeCaPs at the capillary level
3. Development of a low pulsed electromagnetic bioreactor device to eventually control drug release from FeCaPs
4. Realization of a micro-fluidic device to mimic different vessels fluid flow (from aorta to capillaries)
5. Evaluation of FeCaPs stability under different fluid flow regimes

2. Cooperation between participants

CNR-IEIIT-GE collaborated closely with CNR-ISTEC and CNR-IRGB for the achievement of this D2.3, according to the results provided by SIM and CNR-IEIIT-MI in D4.1 (Figure 1).

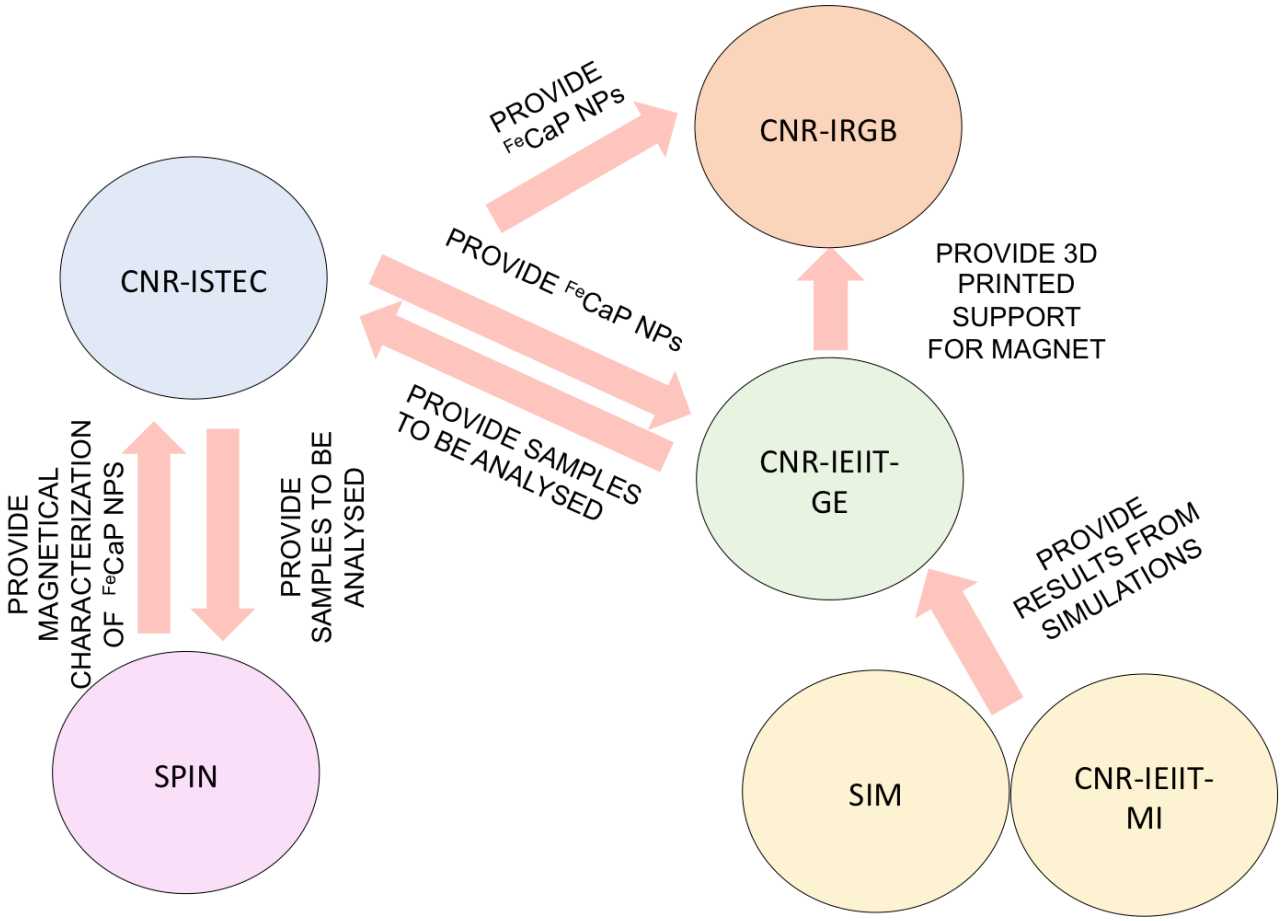

Figure 1. Diagram showing the cooperation between the partners for the progress of this deliverable.

CNR-IEIIT-GE experimentally validated the theoretical results generated with the simulations received from CNR-IEIIT-MI and SIM and related to the combined effects of magnetic and fluidic forces on ^{Fe}CaPs. CNR-ISTEC synthesized and characterized (in collaboration with CNR-IEIIT-SPIN) ^{Fe}CaPs and worked in collaboration with CNR-IEIIT to characterize the physical-chemical features of ^{Fe}CaPs after the applications of the different stimuli. CNR-IEIIT provided CNR-IRGB the printed 3D printed support to perform additional in vitro evaluations about the interaction of ^{Fe}CaP-magnet with cells interaction.

3. In vitro evaluation of ^{Fe}CaP guidance

^{Fe}CaPs have been generated by CNR-ISTEC employing a wet chemical synthesis. Briefly, a $Ca(OH)_2$ aqueous suspension was neutralized with H_3PO_4 in presence of $Fe^{2+/3+}$ ions. Obtained ^{Fe}CaPs displayed a typical superparamagnetic behavior with mass magnetization at saturation (M_S) of 5.0 emu/g and negligible magnetic coercivity, i.e. no magnetic remnance immediately after the removal of the magnetic field. Details of the synthesis process as well as chemical, morphological, and magnetic characterizations performed by CNR-ISTEC and CNR-IEEIT-SPIN are reported in the document related to the D1.3.

For this deliverable, different activities have been performed:

1) Experimental validation of theoretical parameters generated by CNR-IEIIT-MI and SIM and related to the influence of combined magnetic forces and fluidic forces on ^{Fe}CaPs.

2) Generation of a low pulsed electromagnetic bioreactor device to eventually control drug release from ^{Fe}CaPs.

3) Generation of micro-fluidic devices for functionally testing of ^{Fe}CaPs in an *in vitro* platform mimicking different physiological blood velocities.

4) Assessment of ^{Fe}CaPs stability subjected to different physiological velocities and for different time periods.

3.1. Magnetic guiding system

Simulations performed by CNR-IEIIT-MI and SIM, as reported in D4.1, revealed that a static magnetic field applied via a permanent magnet is sufficient for the proper guiding of ^{Fe}CaPs. In particular, the following features have been selected: residual Magnetization: 1.47 T, optimal radius: 5 mm, height >> radius. Following these indications, the appropriate magnets were used by CNR-IEIIT-GE for the proper experimental validation. As part of the generated results, positioning of the magnet close to a plastic tube where ^{Fe}CaPs (0,67 mg/ml) were flowing at the capillary velocity (0.1 cm/s), was sufficient for an effective retention of ^{Fe}CaPs in proximity of the magnet (Figure 2).

Figure 2. The magnet is able to stop the ^{Fe}CaPs from following the fluid flow with the capillary velocity.

In addition to their guidance, ^{Fe}CaPs can be stimulated with a proper electromagnetic stimulus to achieve a controlled drug release. To test this feature, CNR-IEIIT-GE developed a novel electromagnetic bioreactor device and is currently working on its optimization to properly modulate the activity of the superparamagnetic ^{Fe}CaPs. In particular, the application of low-pulsed magnetic stimulations can trigger the release of therapeutic drugs from ^{Fe}CaPs by inducing their mechanical shacking and flipping and thus promoting the drug detachment. Briefly, the bioreactor includes two solenoids (Helmholtz coils) designed to generate the proper magnetic field and associated with a fluidic circuit mimicking the cardiovascular environment. The application of low-pulsed electromagnetic stimulation enhances the efficacy of drug release from ^{Fe}CaPs preserving cardiac electrophysiological proprieties both *in vitro* and *in vivo* (*Paper under submission*).

3.2. Design and fabrication of a micro-fluidic device for *in vitro* tests

CNR-IEIIT-GE has designed and fabricated several micro-fluidic devices to evaluate the combinatory effects of the fluid flow (i.e. Stokes forces) and electromagnetic forces on ^{Fe}CaPs.

The devices were realized through the 3D printing technology by a 3D printer “The Form 2” (Formlabs) with a biocompatible resin (Dental SG Resin). The devices, which are composed by micro-vessels as shown in Figure 3, recapitulate the physiological blood flow velocities of different vessel types: aorta, artery, arterioles, and capillaries.

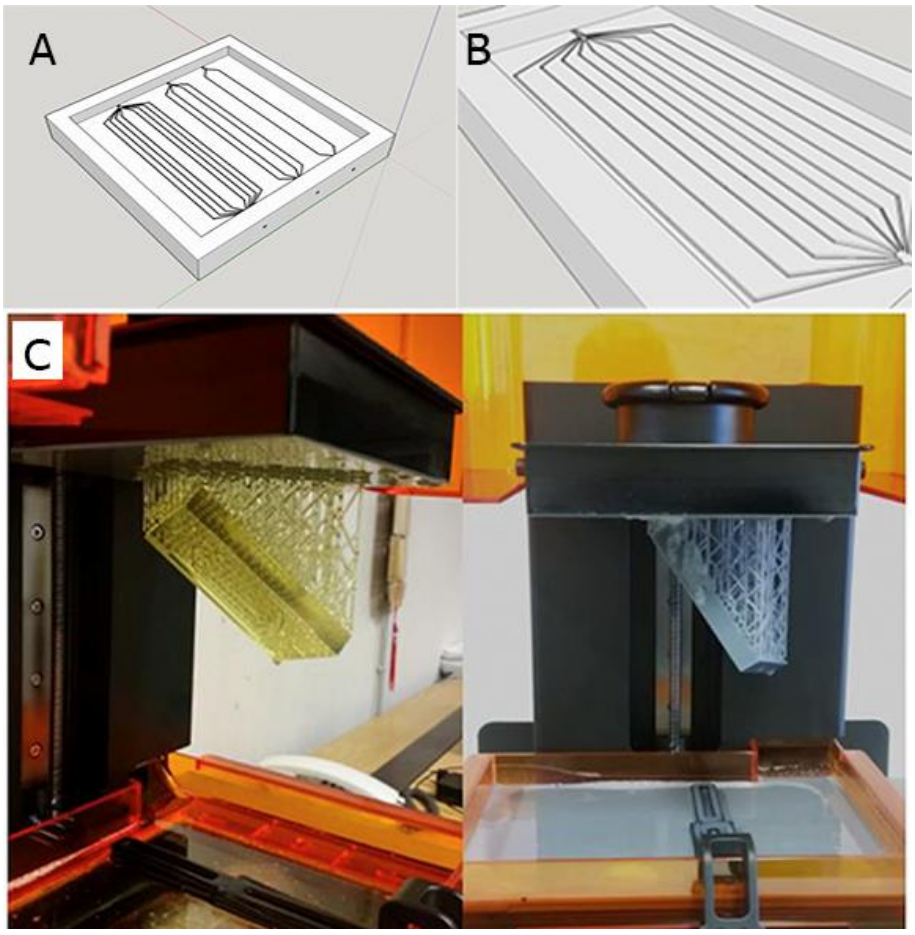

Figure 3. Different phases of the stereolitography 3D printing procedures of the micro-fluidic device. Panels A, B represent the CAD sketches (realized through the Sketchup software (Version 2016)). Panel C shows the device during printing phase.

Designed vessels mimicking aorta, arteries, and arterioles flow velocities as well as branching mimicking capillaries velocities are shown in Figures 4A and B, respectively. Thanks to the designed ramifications of the vessels, and according to the continuity law, different flow velocities among the vessels were simultaneously achieved through the application of a unique inlet velocity (through a peristaltic pump).

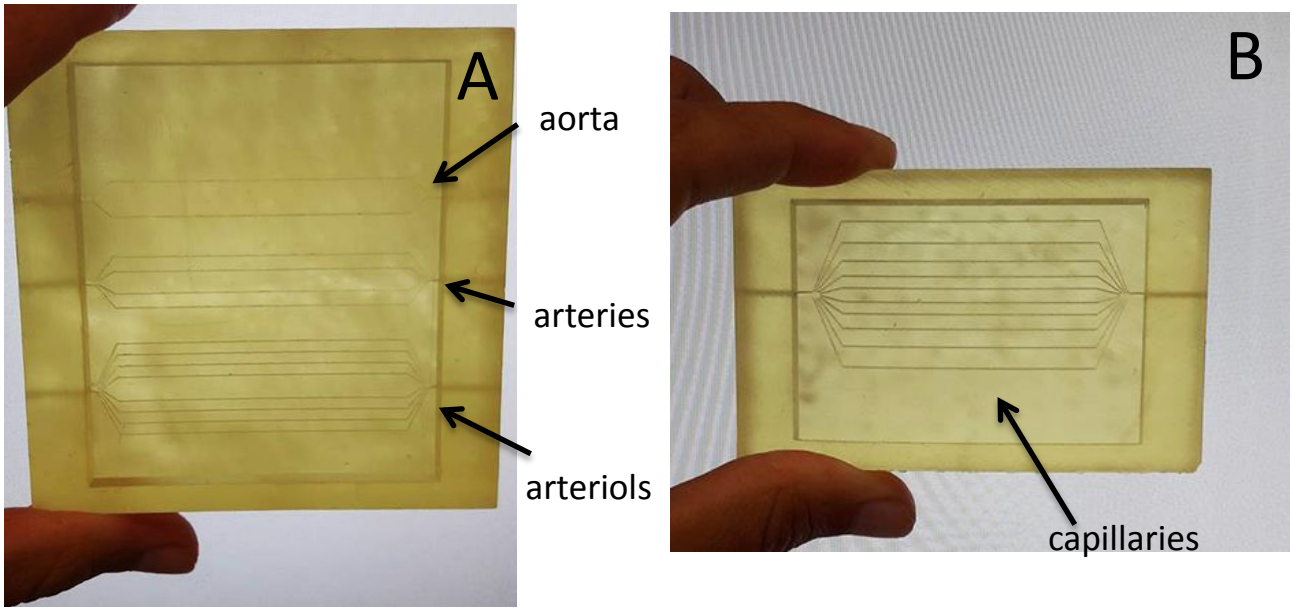

Figure 4. Pictures showing the micro-fluidic devices and their vessels.

To verify the correct printing process and dimension of vessels, each device has been characterized through fluorescence microscopy. In particular, eosin fluorescent solution was injected within the vessels and imaged through fluorescent microscope (Nikon H550L). As shown in Figure 5, the vessels were successfully printed, following the dimensions set during the design process.

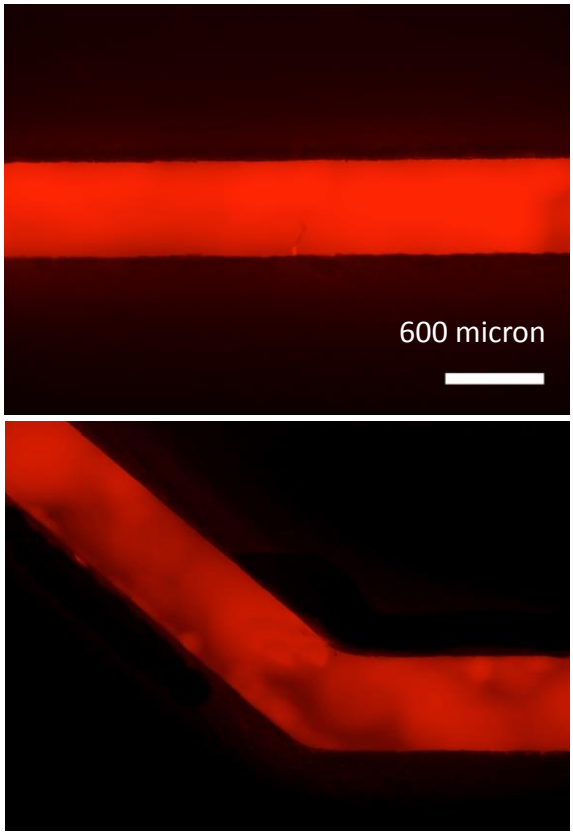

Figure 5. Fluorescent microscopy images of the 3D printed channels

In addition, CNR-IEIIT-GE provided CNR-IRGB with a 3D printed support (Figure 6), which has been designed for additional in vitro evaluations concerning the interaction of ^{Fe}CaP-magnet with cells interaction. Activities are currently ongoing.

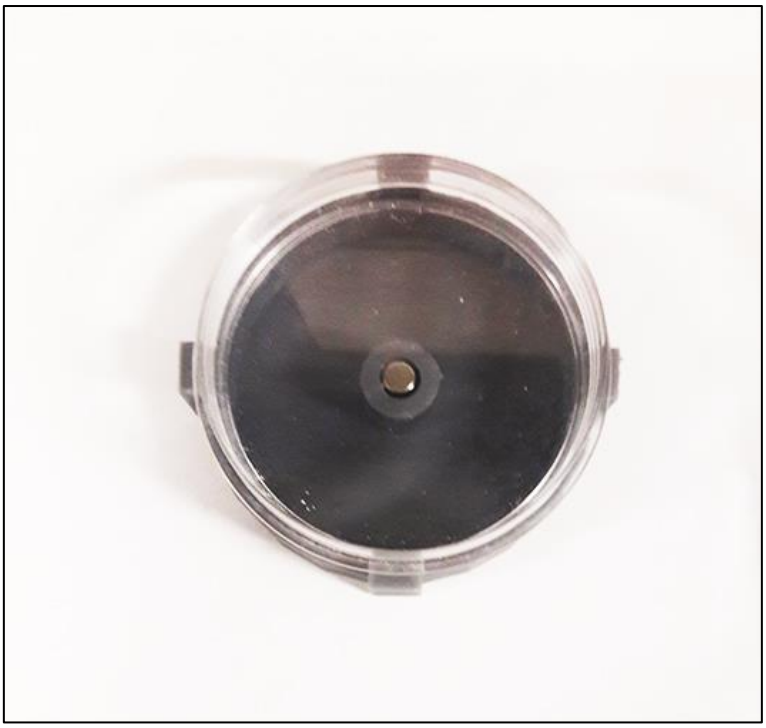

Figure 6. 3D printed support to place the magnet below a petri dishes where to perform cell culture tests.

3.3. ^{Fe}CaPs stability under fluid flow physiological velocities

To identify the best conditions for stability tests, FeCaPs were suspended in different media, namely glucose 5 wt%, NaCl 0.9 wt% and citrate 0.1 M, and the obtained solutions were analyzed by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Ltd., UK). Citrate buffer was found to be the best dispersion medium (data not shown – paper under preparation), giving a narrowed population of FeCaPs with a low polydispersity index and good colloidal stability up to 24 hours.

Thus, to evaluate the FeCaPs stability within the generated device, suspensions of FeCaPs (0,67 mg/ml) in citrate buffer 0.1 M were initially used. Results extracted from these activities show that after different time points (5 minutes for aorta, arteries, arterioles and 5 minutes and 120 minutes for capillaries, respectively), the circulating solution was collected, centrifuged at 12.000 rpm for 15 minutes and filtered (filter pore size: 0,22 micron). The chemical degradation of FeCaPs under different experimental conditions was evaluated as the extent of Ca and P released in the flowing solution by inductively coupled plasma spectrometer (ICP-OES) (Liberty 200, Varian, US) employing wavelengths of 422.673 nm (Ca), 259.940 nm (Fe), and 213.618 nm (P). The results reported in Figure 7 are expressed as a mean of 3 experiments and showed that the FeCaPs dissolution rate is proportional to flow velocity and time, with up to the 60 wt% of FeCaPs being degraded after 5 min under aorta, arteries and arteriols flow condition, and after 2 hours under capillary flow condition. However, it is worth to notice that these experiments were performed using a citrate buffer at pH 6.0 to avoid the formation of FeCaPs aggregates, which are more resistant to dissolution with respect to single nanoparticle. On the other hand, under these conditions the effect of the fluid flow on the dissolution of finely dispersed FeCaPs under slightly acidic condition is maximized; hence, a fast chemical degradation was expected. In this regard, additional experiments will be performed in physiological condition (pH 7.4) to further assess FeCaPs stability. It is expected that in physiological conditions the degradation will be lower.

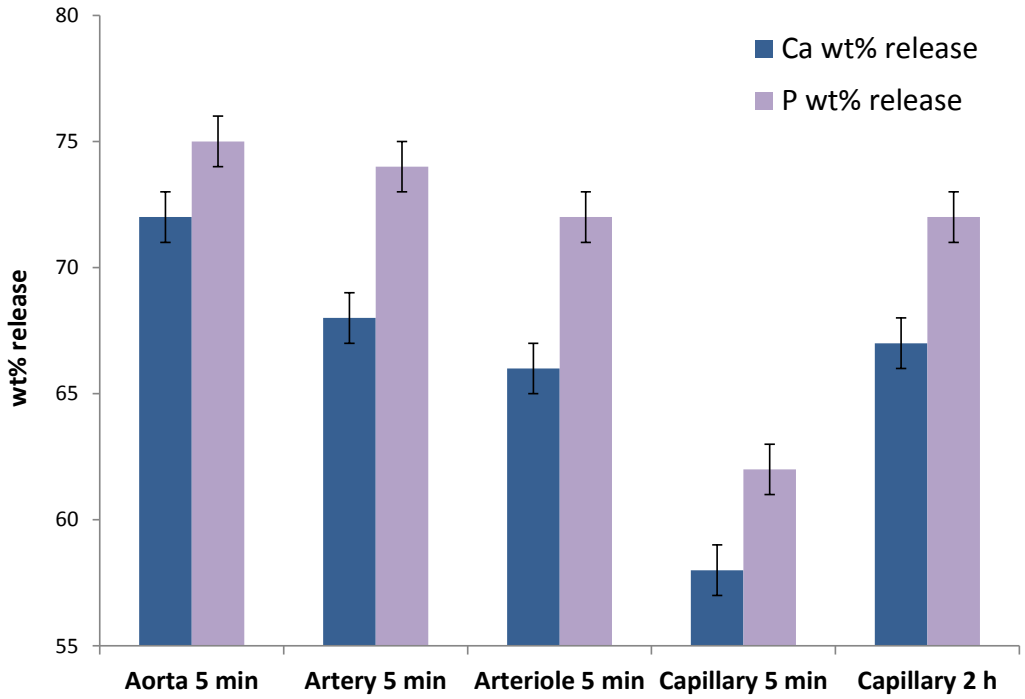

Figure 7. Release mass percentage of Ca and P from ^{Fe}CaP under different flow velocity and times.

4. Conclusions

Results obtained from the theoretical simulations were experimentally validated showing that the static magnet (Br=1,47T) is able to control FeCaPs within the capillary flow.

The application of a low-pulsed magnetic stimulations on FeCaP-loaded with a model drug suspension enhances the efficacy of drug release, which was proportional to the frequency of the applied magnetic field.

The stability of FeCaPs has been evaluated in vitro in a novel 3D printed device able to mimic the fluid velocities of different vessels (from aorta to capillaries). Initial experiments were carried out in a citrate buffer at pH 6.0 to avoid FeCaPs aggregation. Results show that the dissolution rate of FeCaPs is proportional to the fluid flow velocity and time. Further stability tests are currently performed at pH 7.4 to mimic the physiological conditions.

The vessels within the micro-fluidic device will be functionalized with gelatin and coated with endothelial cells to mimic the vessel walls. Experiments wlll be performed to test the interaction and percentage of accumulation of FeCaPs with the functionalized vessels with and without magnet.