PROJECT ACRONYM

CUPIDO

PROJECT TITLE

Cardio Ultraefficient nanoParticles for Inhalation of Drug prOducts

Deliverable 2.4

EMD-mediated guidance of FeCaP

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1. Executive summary

The current deliverable 2.4 outlines a methodology to guide and maximize the time of exposure of nanoparticles to targeted cells employing a magnetic-mediated guidance of superparamagnetic iron doped CaPs (^{Fe}CaPs) (synthesized and characterized in WP1) to the heart by using a magnetic stimulation.

The effects of an external magnetic field on the interaction of ^{Fe}CaP with cardiac cells have been evaluated *in vitro*. In addition, a multi-channel micro-fluidic device working as an *in vitro* platform enable to mimic different physiological blood velocities has been made-up to analyze the interactions of cells with the ^{Fe}CaPs under fluidic stimulation. Finally, *in vivo* experiments have provided the proof of concept for an effective *in vivo* magnet-dependent guidance of ^{Fe}CaPs. Experimental data are partially presented within this public deliverable.

Key deliverable achievements:

1. *In vitro* evaluation of ^{Fe}CaP interaction with cardiac cells under magnetic field stimulation.
2. Realization and validation of a micro-fluidic bioreactor mimicking the fluidic conditions of the blood vessels to be used as an *in vitro* platform for a reproducible and systematic evaluation of ^{Fe}CaP – cell interaction.
3. *In vivo* guidance of ^{Fe}CaPs via external magnet.

2. Cooperation between participants

CNR-IEIIT designed and manufactured two 3D printed devices based on feedbacks and requirements from CNR-IRGB, who is the end-user of the bioreactors. In particular, a 3D printed support for *in vitro* evaluations ^{Fe}CaP-magnet interaction with cardiac cells and a micro-fluidic bioreactor harbouring different fluid flow velocities mimicking the circulatory system.

CNR-ISTEC synthesized ^{Fe}CaPs and transferred them to the other partners.

BIOEMTECH strictly worked with CNR-ISTEC, who provided the know-how for the ^{Fe}CaP synthesis. Thanks to this tight collaboration and personnel visits between the two laboratories, BIOEMTECH optimised the radiolabelling technique of ^{Fe}CaPs. BIOEMTECH performed the *in vivo* biodistribution studies with magnets provided by CNR-IEIIT.

3. *In vitro* EMD-mediated guidance and stimulation of ^{Fe}CaP

CNR-IEIIT outlined a methodology and the relevant devices to design a magnetic-mediated guidance of superparamagnetic iron doped CaPs (^{Fe}CaPs) (synthesized and characterized in WP1) to the heart. This strategy is alternative (or even complementary) to the chemical approach employing myocardial-specific internalizing aptamers that can overcome any potential limitation due to the lack of a cell-specific receptor at the heart level.

Firstly, the activities were directed towards the experimental validations of results obtained from the theoretical simulations generated in WP4 and related to the interaction between magnetic forces and fluidic forces. By using a static magnet featuring the required properties, data have been experimentally confirmed. Successively, several micro-fluidic devices have been made by using 3D printing technology to be used as *in vitro* platforms to mimic different physiological blood velocities. In these settings, the stability of ^{Fe}CaPs exposed to several blood velocities (from aorta to capillaries ones) mimicking the physiological stream, has been assessed over time. Moreover, another 3D printed bioreactor prototype has been properly designed and made-up to monitor, *in vitro,* the cellular interactions with NPs in a circuit mimicking the blood fluid flow. In addition, a low-pulsed electromagnetic bioreactor device has been developed and tested to control drug release efficacy from ^{Fe}CaPs.

Part of the activities in this Deliverable resulted in a publication (Marrella et al., J R Soc Interface. 2018).

Magnetic stimulation system

Simulations performed by CNR-IEIIT (Milan) and SIM, as reported in WP4, revealed that a static magnetic field applied via a permanent magnet is sufficient for the proper guiding of ^{Fe}CaPs. Therefore, the appropriate magnets were used by CNR-IEIIT (Genoa) for corresponding experimental bench validation. Taking into consideration the experimental results from the above tests, CNR-IRGB, performed the subsequent ^{Fe}CaP biocompatibility assays (data not shown, refer to Marrella et al., J R Soc Interface. 2018), evaluated and confirmed the internalization of ^{Fe}CaP in cardiac cells and the increased uptake dependent on the application of the external magnetic field.

Micro-fluidic bioreactor

CNR-IEIIT has designed and made-up via 3D printing technology a new prototype of micro-fluidic bioreactor for cellular *in vitro* testing. In particular, the purpose of the system is to analyze the interactions of endothelial cells with ^{Fe}CaPs under fluidic stimulation. The modelling of the bioreactor was based on the approximation of several theoretical models. Multiple channels were included where the culture media circulates with the same velocity. As such, different velocity fields (from capillaries to aorta) can be achieved by simply changing the input flow rate set in the peristaltic pump. The micro-channels of the bioreactor have been tested with human dermal microvascular endothelial cells (HMEC-1).

Electromagnetic bioreactor device

In addition to their guidance, ^{Fe}CaPs can be stimulated with an appropriate low-frequency electromagnetic stimulus to achieve a controlled drug release (see data reported by Marrella et al., J R Soc Interface. 2018) (Figure 1). CNR-IEIIT (Genoa) developed an electromagnetic bioreactor to generate an optimizedmagnetic field at the level of a fluidic circuit mimicking the cardiovascular environment (Figure 1, exp design 1). It was demonstrated that the device was able to trigger the release of a model drug (ibuprofen) from ^{Fe}CaPs as a function of the applied frequencies. The effects on the cardiac system of the identified electromagnetic exposure were assessed *in vitro* and *in vivo* by stimulation of isolated adult cardiomyocytes and in an animal model. The cardio-compatibility of ^{Fe}CaPs was assessed *in vitro* and in animal model. No alterations of cardiac electrophysiological properties were observed, providing the evidence that the combination of low-frequency magnetic stimulations and ^{Fe}CaPs represents a promising strategy for controlled drug delivery to the failing heart.

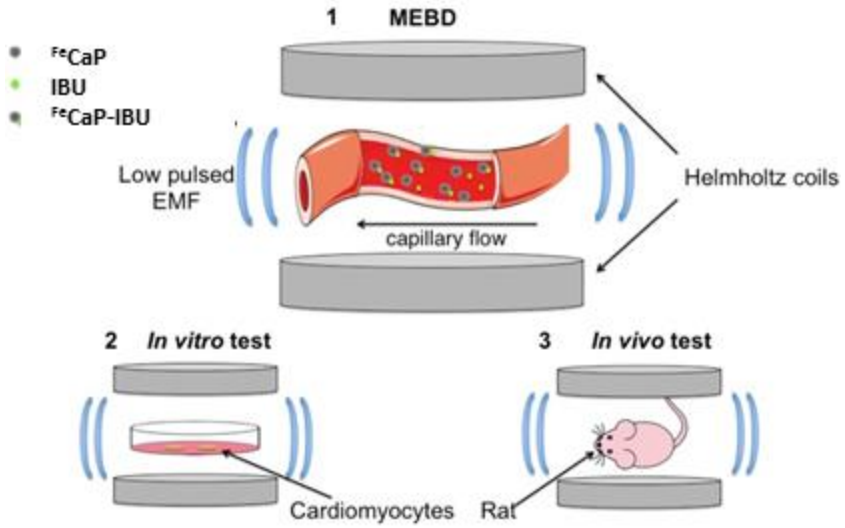

Figure 1. Schematic representation of the electromagnetic bioreactor device (MEBD) (1), *in vitro* (2) and *in vivo* (3) tests have been performed to evaluate the biological effects on the cardiac system of the electromagnetic exposure (see data reported by Marrella et al., J R Soc Interface. 2018).

4. *In vivo* magnetic guidance of ^{Fe}CaP

In vivo experiments performed in healthy Swiss albino mice (Figure 1, exp design 3) supported the effective guidance of ^{Fe}CaP via an external permanent magnet. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Further studies are currently on going for deeper evaluation of the system.

5. Conclusions

A methodology to maximize the time of exposure of nanoparticles to cardiac cells employing a magnetic-mediated guidance of superparamagnetic iron doped CaPs (^{Fe}CaPs) has been developed. After assessing that a static magnet is able to guide ^{Fe}CaPs circulating in a capillary flow and demonstrating that 80% of ^{Fe}CaPs are stable in fluidic conditions, the effects of the external magnetic field on the interactions between the NPs and cardiac cells is now being evaluated.

A micro-fluidic bioreactor, aiming to evaluate the NPs interaction with endothelial cells under different fluid flow velocities of the circulatory system, has been manufactured and experimentally validated.

In vivo experiments on healthy mice with an external magnet, placed on the animal's skin, have demonstrated the ability *to in vivo* guide the NPs to selected target regions.