Scientific report on the implementation of the project

PN-III-P1-1.1-PD-2016-1642 (contract no. 110 / 2018) entitled *Development of a multifunctional radiolabelled hybrid magnetic nanoparticle agent for dual-modality (SPECT and MRI) medical imaging*

(Acronim: NANOIMAG)" Phase 3-2020

Phase 3: Multimodal imaging evaluation of the obtained nanotracer using SPECT and MRI and comparison studies with fluorescent/PET on small animals imaging

Related delivered activities:

A5.1. Scintigraphy biodistribution studies

A5.2. Small-animal MR imaging

A5.3. Fluorescent/PET imaging on small animals

A5.4. Comparison studies

During the phase 3-2020, 1 type of hybrid nanoparticles were prepared, namely magnetite nanoparticles obtained by co-precipitation of iron ions, subsequently coated with 3-aminopropyl triethoxysilane (batch GD 17) and tested as an imaging nanotracer for SPECT and MRI;

The hybrid magnetic nanoparticles were the ones prepared using magnetic core (iron oxide) and multifunctional silica shell chain (with 3- aminopropyltriethoxysilane) and glucose using very simple methodology (Figure 1).

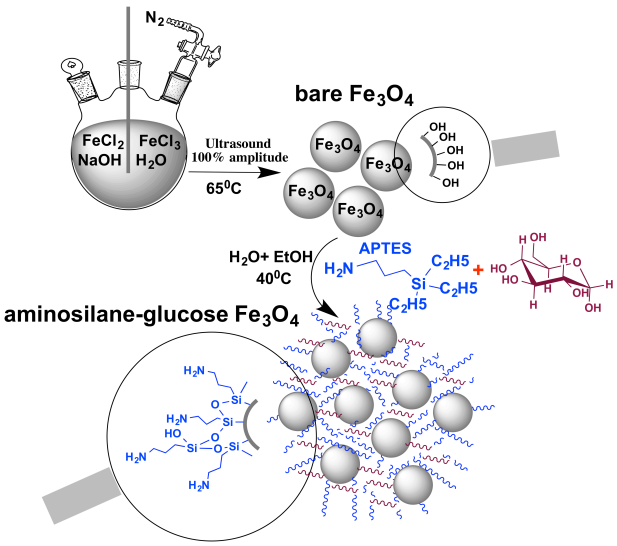


Figure 1. Reaction method

The nanoparticles were radiolabelled with $^{99m}TcO^{-}_{4}$ Na (sodium pertechnetate) chosen for labelling because it is the most commonly used emitting radioisotope in nuclear

medicine having a convenient half-life of approximately 6 h, appropriate energy (140 keV) for imaging on a standard gamma camera and less attenuation by soft tissue. For optimum labelling yield, many factors were studied in details such as, the variation of the reducing agent (sodium borohydride and/or stannous chloride) and concentration, the variation of the Na^{+} $^{99m}TcO_4^{-}$ dose used and reaction time. The radiolabelling efficiency was assessed by using instant thin layer chromatography (ITLC). The coupling stability of the radiolabeled hybrid magnetic nanoparticles was verified in both sterile water and human serum at room temperature at different intervals (1, 2, 3, 4, 5 and 6 hours) as presented in Figure 2.

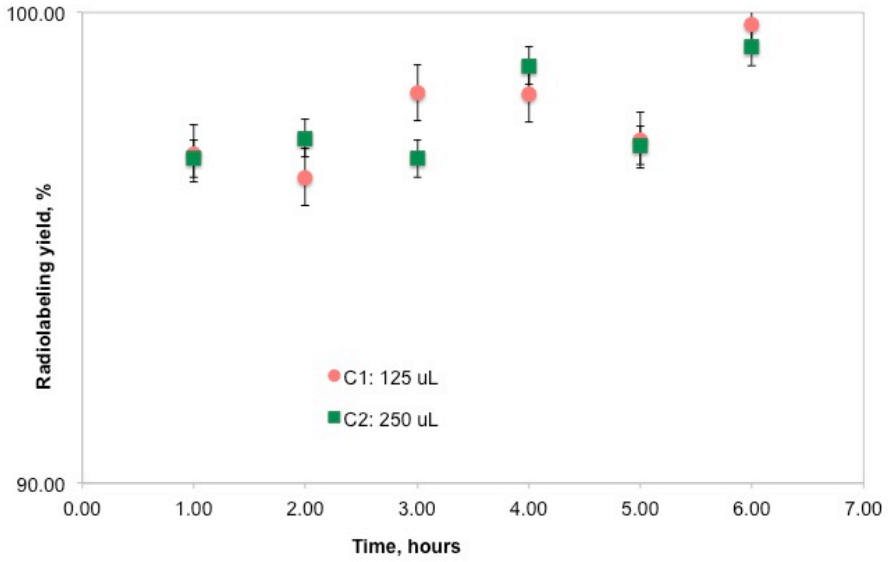
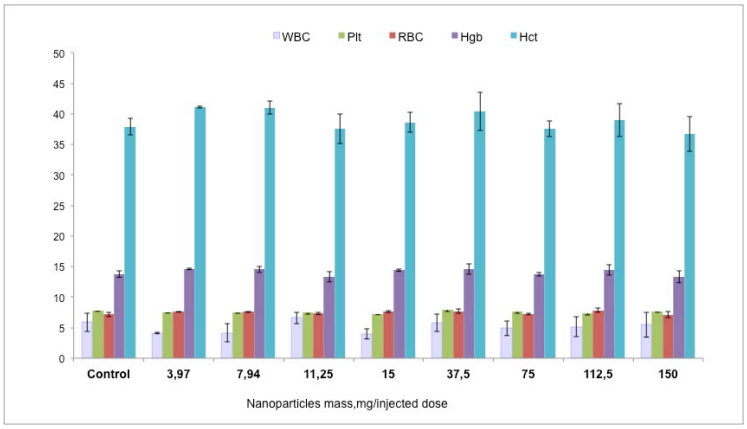


Figure 2. Radiolabelling yield for magnetic amine nanoparticles (batch GD17).

The cytotoxicity induced by hybrid nanoparticles was investigated by MTT assay at 6, 12, 24 and 48 hours.

All animals experimental procedures employed in the present study are strictly in accordance with the European Community Guidelines regarding ethics and approved by ‘‘Grigore T. Popa’’ University of Medicine and Pharmacy animal care and use committee.

Toxicity: The agents under study were administered and then, carefully observed for obvious signs of toxicity, such as convulsions or body weight effects or by macroscopic evidence of organ and tissue damage or dysfunction.



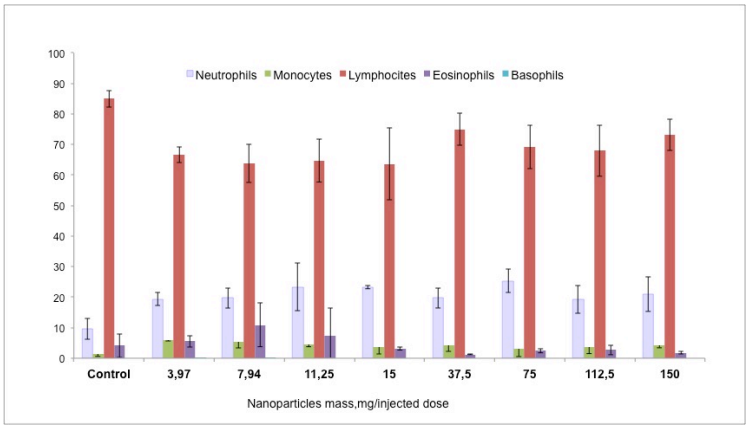
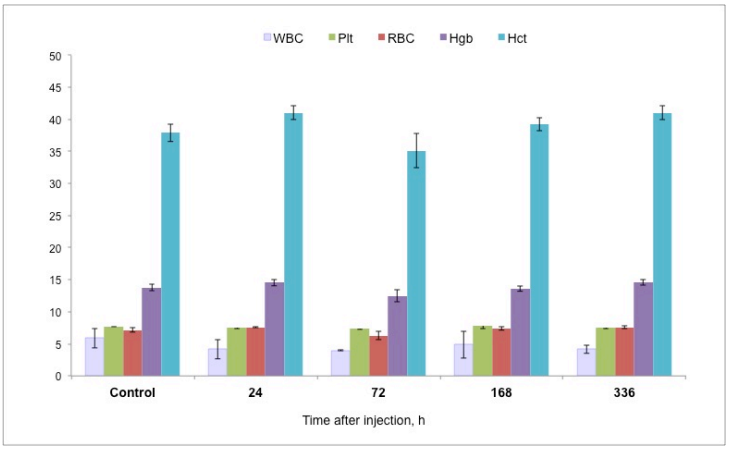


Figure 3. In vivo toxicity assessment: the short-term (acute) toxicity



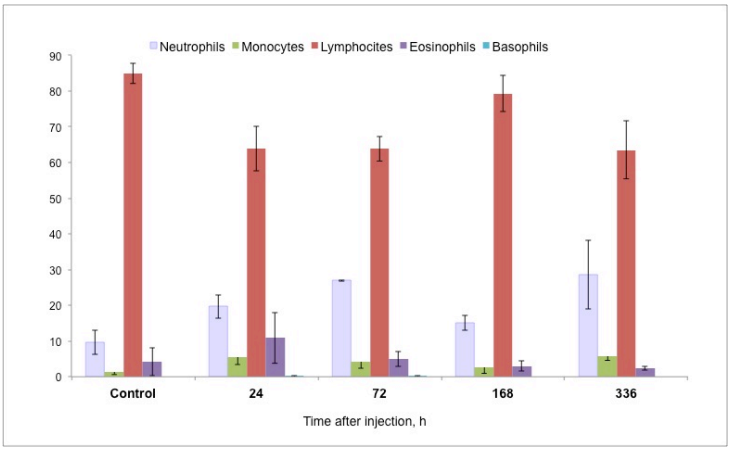


Figure 4. ***In vivo* toxicity assessment:** the long-term (chronic) toxicity

Scintigraphy biodistribution studies: distribution studies on normal tissues using gamma counter. Estimation of the possible separation of free ^{99m}Tc/degradation after administration of labelled complexes by ITLC strips blood analysis (Figure 5).

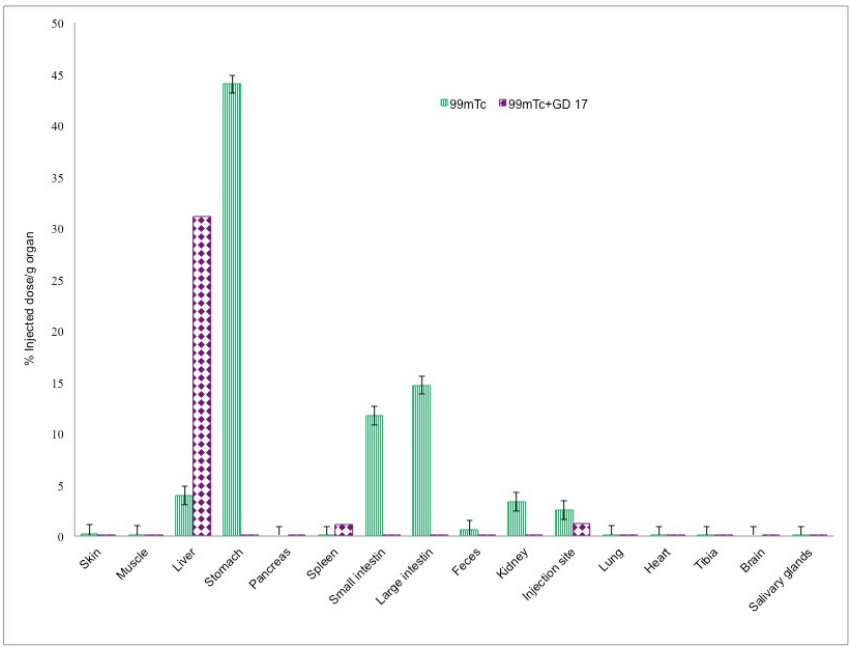


Figure 5. Ex vivo biodistribution of radioactivity after the injection of ^{99m}Tc and radiolabeled aminosilane nanoparticles (^{99m}Tc+GD17 batch) at 6 h.

Small-animal MR imaging:

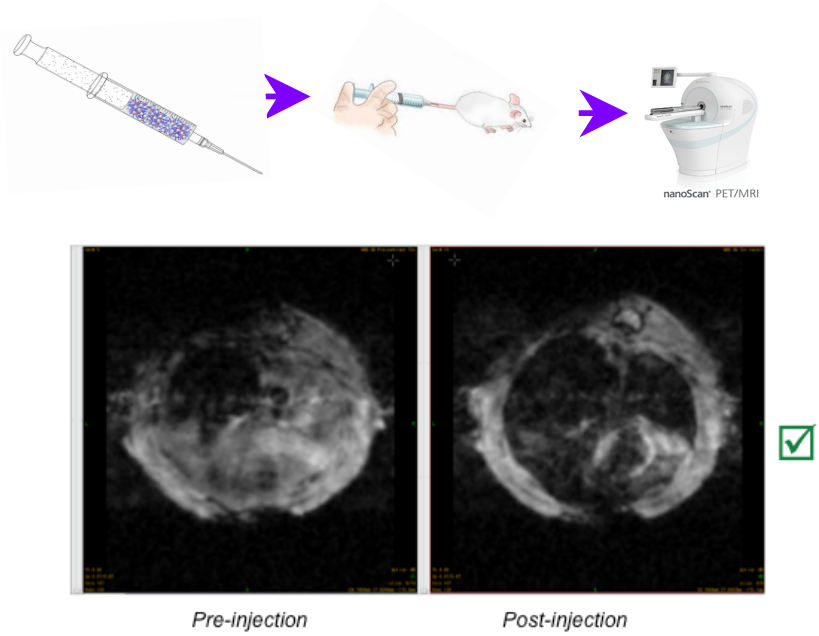


Figure 6. In vivo MRI biodistribution of the obtained hybrid magnetic nanoparticle probe on healthy experimental animals: GRE T2* sequence

Significant MRI signal change was observed over time on: liver (ex. 39% at 60 minutes, 50% at 260 minutes, 33% at 570 minutes), aorta, cava vein and hepatic vein.

Further studies must be performed to quantify the observed changes.

Conclusions phase 3

The deliverables associated with each activity that were obtained:

- Dual-modality (SPECT and MRI) medical imaging agent;
- Oral presentation at 27th Young Research Fellows meeting, 29-31st January 2020 at University of Caen Normandie, Caen, France: Magnetic hybrid nanoparticles: facile preparation, toxicity and MRI biodistribution by G. Dodi, C.M. Uritu, C.T. Mihai, M. Furcea, L. Agrigoroaie, A. Starica, A. Diaconu and I. Gardikiotis;
- Review paper: Nanomaterials Designed for Antiviral Drug Delivery Transport across Biological Barriers, by F.-D. Cojocaru, D. Botezat, I. Gardikiotis, C.-M. Uritu, G. Dodi, L. Trandafir, C. Rezus, E. Rezus, B.-I. Tamba, C.-T. Mihai; Pharmaceutics 2020, 12, 171;
- 1 paper sent for publication: Preparation, characterization and biocompatibility studies on magnetite nanoparticles: Mechanical stirring vs. high-pressure homogenization vs. ultrasound, by G. Dodi, V. Balan, C.M. Uritu, T. Cacciaguerra, C.T. Mihai, A. Rotaru, I.L. Serban;
- 1 activity report.