Final Report

Annex I

Project No: 327151

Project Acronym: Magnetic_PCR

Project Full Name: Magnetic-PCR: An ultrasensitive methodology for Breast cancer detection and characterization.

1. FINAL PUBLISHABLE SUMMARY REPORT

Summary description of the project objectives

The Magnetic-PCR project proposes a brand-new and ultrasensitive methodology for detection and classification of breast cancer subtypes based on detection of specific genes expression. For this, the project develops new "magnetic relaxation switch" (MRSw) biosensors based on superparamagnetic nanoparticles to improve the sensitivity of the Polymerase Chain Reaction (PCR) for detecting nucleic acids. Relaxometry and Magnetic Resonance Imaging (MRI) have been used to sensing DNA or RNA biomolecules. The ultimate goal is to achieve direct application of a MRSw sensor for detection and classification of breast cancer malignancy by magnetic resonance (MR). The advantages of magnetic relaxation switch (MRS) methodology for point-of-care assays include its use of radiofrequency radiation rather than light (indifference to light based interferences), and its use of solution phase chemistry (no solid phase, no separation of free and bound as in ELISA's).

Description of the work performed since the beginning of the project

The main focus of this grant have been the development of new methods for the ultrasensitive detection and identification of breast cancer subtypes, **improving cancer classification and chemotherapy**. The proposed research is highly interdisciplinary. Nanoparticle synthesis and surface chemistry have been employed to obtain nanoparticles recognizing specific sequences of DNA that define the breast cancer subtype. MR relaxometry, MRI, and the related physics, have been employed to measure nanoparticle aggregation state as the spin-spin relaxation time of water protons. Cancer biology and molecular imaging techniques have been employed to obtain, characterize and quantitate several cancer cell lines.

During the period of the project (2013-2015), our first objective was the development of magnetic nanoparticles for specific DNA sequence detection by PCR and MRI and optimize nanoparticle design. Though nanoparticle aggregates are affected by a number of factors, including DNA surface density, nanoparticle size, interparticle distance, and salt concentration, the effect of oligonucleotides length in the speed of the aggregation process within magnetic nanoparticles have never been studied. **Design factors evaluated** have been: (i) selection of oligonucleotide as polymeric backbones for the bases G, C, T and A; (ii) selection of the optimum polymer length (number of bases); (iii) selection of the conjugating reagent (linkage to nanoparticle); (iv) selection of the optimal size, shape and composition of the magnetic NPs.
In the second part of the project our research was focused on the development of a protocol for real time-PCR (rt-PCR or qPCR) with magnetic nanoparticles.

Description of the main results achieved so far

The **Magnetic-PCR project** has proved the application of MRSw methodology for quantification of PCR produced DNA by relaxometry measurements. Our initial approach targeted a MNP functionalized with oligonucleotides (primers of selected breast cancer genes) on its surface by using the carbodiimide/NHS chemistry set. qPCR showed good performance as quantified by fluorescence (Fig. 1) but there were few aggregation processes involved and, therefore, the deltaT2 values obtained by relaxometry were not very significant, with ΔT2 values around 10 ms (ΔT2 = /T2 initial - T2 final/).

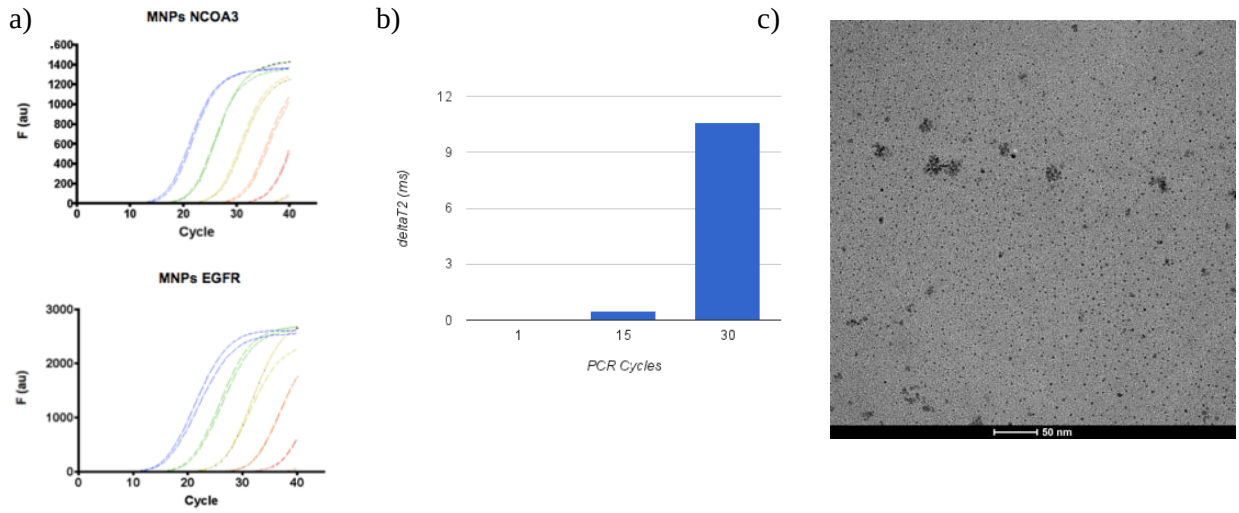

Figure 1. a) qPCR performance analysis of regular PCR reaction with MNPs PCR reaction for NCOA3 and EGFR genes at several DNA concentrations (1uM to 0.1fM); b) deltaT2 values obtained from NCOA3 gene PCR reaction with MNPs; c) TEM micrograph of PCR reaction showing MNPs clusters in low proportion.

After numerous negative results and long processes of optimizing the methodology and changes in the synthetic procedures, we were able to obtain a MRSw with good sensitivity and selectivity. These MRSws showed ΔT2 values around 25ms and aggregated forming big size clusters (Fig. 2).

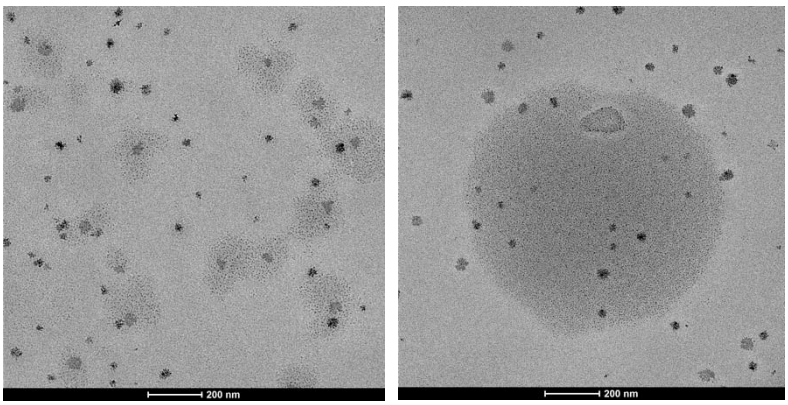

Figure 2. TEM micrographs of qPCR reactions aggregates with thiolated-primers MNPs; 3 types of aggregates were found.

Expected final results and their potential impact and use

Magnetic-PCR project succeeded in designing a stable magnetic nanoparticles platform for amplifying selected breast cancer genes within PCR and qPCR reactions. The success was achieved by combining three principles: (i) sequence-specific DNA binding surface design, (ii) multivalency enhanced, solution phase, microaggregate formation when NP's oligonucleotides are amplified by PCR and, (iii) detection of NP/DNA microaggregates by MR. These three principles were combined to obtain a highly sensitive general method of detecting, without risk of post-amplification contamination, the DNA generated by the PCR reaction.

We also developed robust protocols for PCR analysis with magnetic nanoparticles with the ultimate goal of achieving direct application of MRSw sensors on the detection and classification of breast cancer malignancy by magnetic resonance. Due to the complexity of the project, in vitro and in vivo analysis could not be performed (as initially planned). These assays are required for a successful translation of the project to clinics and to be applied in high throughput screenings (clinical microbiology, virology, hematology, etc.).