Magnetic Nanoparticles for Biomedical Applications

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Magnetic nanoparticles (MNPs) are crucial and extensively studied for various biological applications due to their manipulability *via* external magnetic fields. For instance, they can be guided by a magnetic field gradient towards specific regions within the body. Additionally, when exposed to an alternating current (AC) magnetic field, they dissipate heat through energy loss mechanisms, with hysteresis being a significant contributor.

A promising area of application for MNPs in oncology is magnetic hyperthermia, a therapeutic technique often used as an adjuvant in cancer therapy, particularly in radiotherapy and chemotherapy. This therapeutic approach involves the use of MNPs introduced into the tumor region and subsequently activated by an AC magnetic field. This activation induces the controlled release of heat, aimed at raising the temperature in the tumor area by approximately 4-5 °C, making cancer cells more sensitive to pharmacological and radiotherapy treatments. A significant aspect of magnetic hyperthermia is its selectivity, as healthy cells are less thermosensitive compared to cancer cells. In particular, solid tumors possess a complex vascular architecture, including hypoxic and low-pH regions absent in normal tissues, rendering cancer cells more vulnerable to temperature elevation.

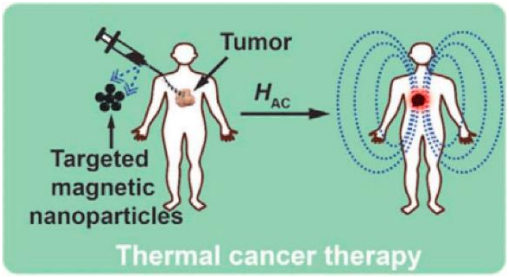

Figure 1. Schematic representation of the magnetic hyperthermia mechanism. This image is extracted from http://dx.doi.org/10.3390/magnetochemistry5040067

This thesis aims to develop MNPs with high thermal efficiency and to employ the generated heat for various applications, including magnetic hyperthermia on cell cultures, magnetically mediated drug delivery, drug-controlled release by thermosensitive nanocarriers, and the induction of acrylamide radical polymerization to produce hydrogels.

Synthesis and characterization of magnetic nanoparticles with surface coating

The co-precipitation synthesis is employed to obtain magnetite (Fe_3O_4) and cobalt ferrite ($CoFe_2O_4$) NPs. After the synthesis, these MNPs are functionalized with different coating agents to improve their colloidal stability and heating efficiency. The synthesized MNPs are thoroughly characterized to determine their dimensional, physicochemical, magnetic, and heating properties. The effectiveness of the surface coatings is assessed through comprehensive characterization, comparing the thermal properties of untreated and treated MNPs to evaluate the effect of the coating. The results show that citrate coating significantly enhances the thermal properties of MNPs, which are expressed through the specific loss power (SLP) parameter. In particular, SLP quantifies the thermal power dissipated per unit mass of magnetic material.

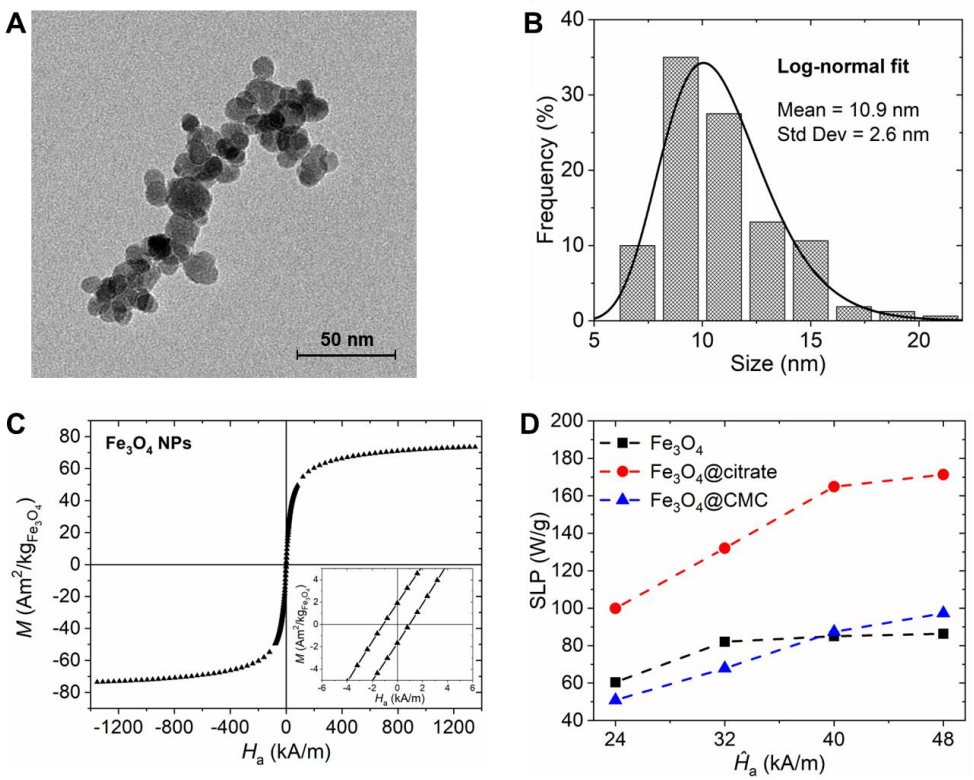

Figure 2. (**A**) Representative TEM image and (**B**) size distribution histogram (derived from statistical analysis on TEM images) of Fe_3O_4 NPs. (**C**) Room-temperature M(H) curves of Fe_3O_4 NPs in dry form. (**D**) SLP values for uncoated and coated Fe_3O_4 NPs obtained by thermometric characterization (AC magnetic field with peak amplitude in the range 24-48 kA/m and frequency of 100 kHz). SLP values of citrate-coated NPs (red) show enhanced heating efficiency compared to uncoated NPs (black).

In vitro experiments of magnetic hyperthermia

The biocompatibility and potential cytotoxic effects of Fe_3O_4 and $CoFe_2O_4$ NPs at different concentrations, both with and without citrate coating, are evaluated through *in vitro* cytotoxicity tests. Fe_3O_4 NPs exhibit significantly high biocompatibility, in particular for lower concentrations (see Fig. 3A). Moreover, they show a higher cellular viability compared to $CoFe_2O_4$ NPs.

Additionally, a novel protocol for conducting *in vitro* magnetic hyperthermia experiments using Fe_3O_4 NPs on adherent cultures of a human lung cancer cell line is developed. This protocol aims to assess the effects of heat generated by internalized nanoparticles or those in close contact with cell membranes, aiming to better mimic physiological conditions. Results from this experiment show effective cell mortality induction, with a greater reduction in cell viability observed for the Fe_3O_4 sample with citrate coating (see Fig. 3B). This aligns with the higher SLP value for this sample, indicating enhanced efficacy in reducing cell viability through magnetic hyperthermia.

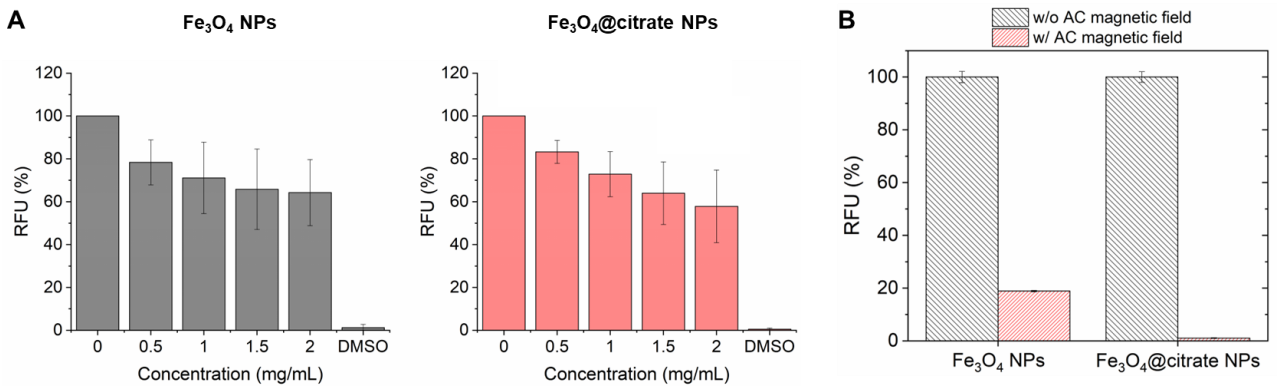

Figure 3. (**A**) Cell viability assessment on A549 cell cultures after 72 h of incubation with four different concentrations (0.5-2 mg/mL) of uncoated and citrate-coated Fe_3O_4 NPs. The data reported are normalized to the control (without NP treatment), which is considered 100%. (**B**) Cellular viability assessment on A549 cell cultures after 72 h from magnetic hyperthermia treatment induced by MNPs internalized within the cellular cytoplasm or closely associated with cell membranes. The data reported are normalized to the control (cells treated with MNPs but not exposed to the AC magnetic field), which is set at 100%. Cell viability is evaluated using the resazurine assay.

Magnetic oxygen-loaded nanodroplets

Oxygen-loaded nanodroplets (OLNDs) are nanocarriers designed to transport and release oxygen for drug delivery applications, i.e. in the treatment of hypoxic tissues. These nanovectors are based on a fluorocarbon core to store oxygen molecules and a polysaccharide coating to provide stability to the nanostructrure. In the literature, OLNDs are activated through ultrasound (US) fields to induce oxygen release.

In this work, OLNDs are magnetically decorated with Fe_3O_4 NPs, resulting in magnetic oxygen-loaded nanodroplets (MOLNDs). The purpose of the magnetic decoration is to create dual-responsive nanosystems (responsive to both US fields and AC magnetic fields) for the controlled release of oxygen. Four samples with varying compositions are synthesized and characterized in terms of their dimensional and morphological properties. Characterization results confirm successful magnetic decoration and other experiments are performed to evaluate oxygen release induced by AC magnetic field exposure. Moreover, *in vitro* cytotoxicity tests are also conducted to assess the biocompatibility of these nanohybrid systems.

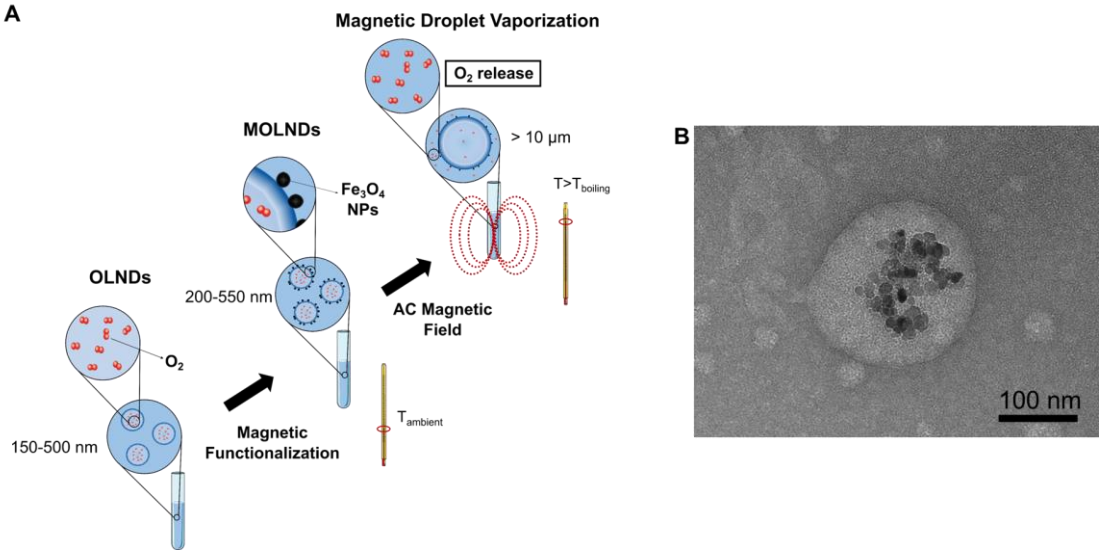

Figure 4. (**A**) Schematic representation of the magnetic decoration of oxygen-loaded nanodroplets with Fe_3O_4 NPs, followed by activation via AC magnetic field, leading to oxygen release through magnetic droplet vaporization. (**B**) Representative TEM image of a magnetic oxygen-loaded nanodroplet. In this image, an aggregate of MNPs is visible on the surface of the droplet.

Application of magnetic hyperthermia in radical polymerization processes

An innovative method, exploiting the thermal energy released by MNPs under the influence of an AC magnetic field, to induce radical polymerization of magnetic hydrogels is presented. Radical polymerization is important in the field of materials science, offering a versatile way to engineer materials with precise properties and responsiveness. Specifically, the utilization of $CoFe_2O_4$ NPs to polymerize polyacrylamide hydrogels represents a good alternative to conventional techniques to promote radical polymerizations, i.e. thermal heating or light irradiation.

Magnetic hyperthermia appears also as a versatile tool for controlling temperature within confined regions of the sample, a notable example is its application in selective volume polymerization within a thermo-sensitive environment. Furthermore, these findings set the stage for the production of non-magnetic materials, wherein $CoFe_2O_4$ NPs are initially concentrated in a small solution volume by a permanent magnet, subsequently facilitating the activation of polymerization across the entire material by an AC magnetic field.

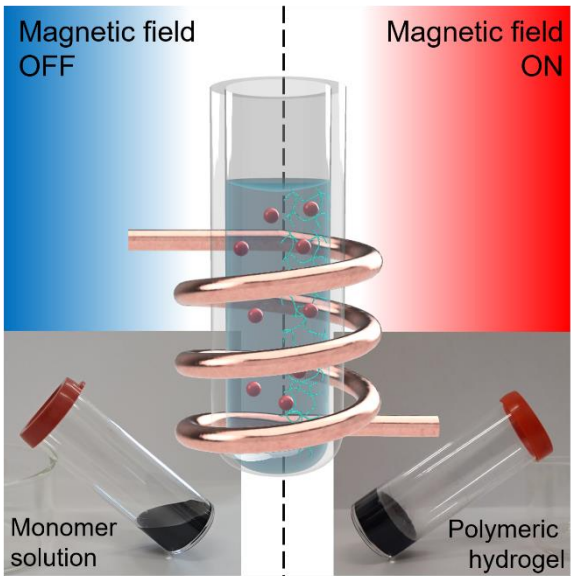

Figure 5. Schematic representation of radical polymerization induced by heat released from $CoFe_2O_4$ NPs, activated by an AC magnetic field.