Multifuctional Nanoparticles for Cancer Theranostic

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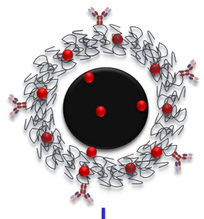
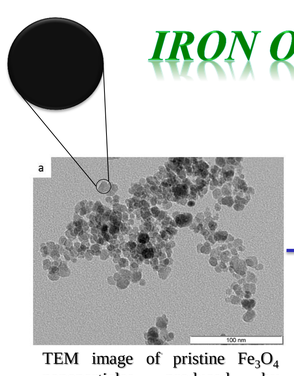
Recent advances in nanotechnology have driven the development of multifunctional nanoparticles (NPs). Multifunctional NPs for cancer theranostic have several potentialities such as drug delivery, specific targeting, treatment, diagnostic or treatment monitoring. Iron oxide nanoparticles are having been extensively investigated for several biomedical applications such as hyperthermia and magnetic resonance imaging for cancer treatment.

A novel approach for cancer theranostic includes multifunctional NPs composed of a magnetite core (iron oxide), coated with a chitosan derivative, incorporating a anti-cancer agent and grafted with a monoclonal antibody anti-CA IX specific for tumor cells. The mechanism of action of these NPs include: (1) specific targeting through the monoclonal antibody; (2) killing cancer cells by hyperthermia; (3) drug delivery of the anti-cancer agent; (4) diagnostic and treatment monitoring through MRI.



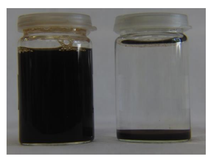
IRON OXIDE NP CHARACTERIZATION



TEM image of pristine Fe_3O_4 nanoparticles produced by chemical precipitation.

Problem

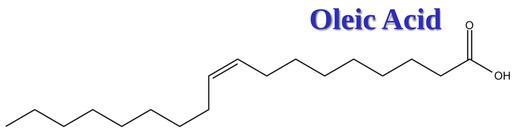
Unstable in aqueous solution

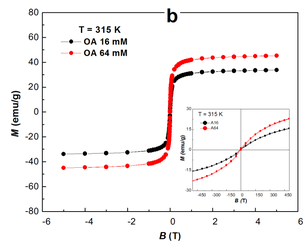


Solution

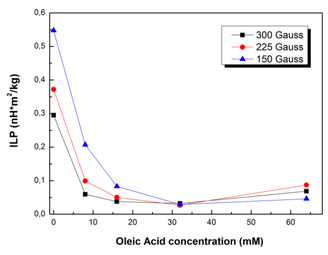
Coating with surfactants

Oleic Acid



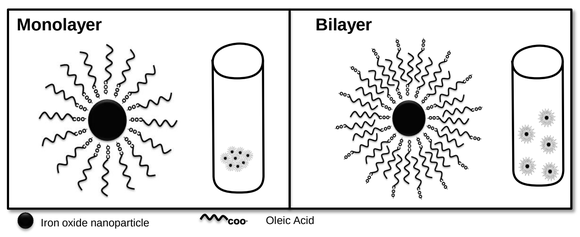


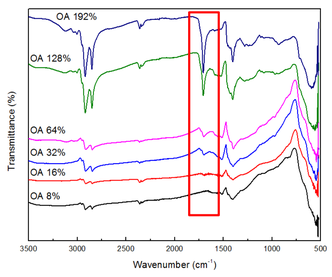
Magnetic Characterization of iron oxide NPs coated with oleic acid 16% and 64%. It is visible the lower concentration of oleic acid has a higher influence in the magnetic susceptibility of the NPs.



Magnetic hyperthermia of iron oxide NPs coated with different concentrations of oleic acid. It is visible that above 20% of oleic acid the ILP has a slight increase.

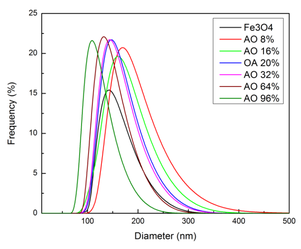
OA forms a bilayer above a concentration of 20%

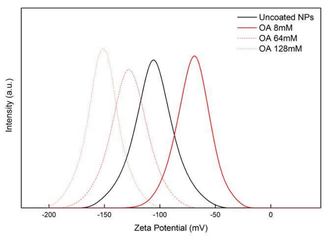




FTIR spectra of iron oxide NPs coated with different percentages of oleic acid (OA) showed that above 32% a sharp band at 1704 cm^{-1} appears, which corresponds to the stretching vibration of C=O in OA, while the peaks between 1550 and 1450 cm^{-1} tend to disappear. Considering that OA links to the Fe_3O_4 NP surface through the carboxylic group, when OA forms a monolayer, only the COO^- group appear on FTIR spectra. However, with the addition of more OA, the COOH group starts to become visible in the FTIR spectra, thus justifying the formation of the bilayer.

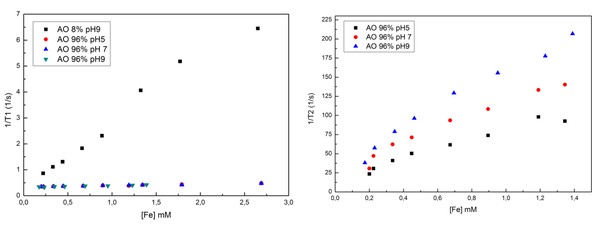
Dynamic light scattering measurements showed that the higher the concentration of OA, the smaller the hydrodynamic diameter of the NPs. This fact may be justified by the increase of stability of the iron oxide colloids, leading to less aggregation and smaller diameter in aqueous solution.





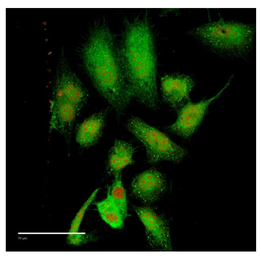
Uncoated iron oxide NPs have a mean zeta potential of -105.2 mV. With lower concentrations of OA (8%) some of the negative surface charges of the NPs are neutralized by the carboxylic group OA, and the zeta potential becomes less negative. For higher concentrations of OA, zeta potential becomes more negative, which indicates that the carboxylic groups of the surfactant are at the surface of the NPs, therefore creating a bilayer

The oleic acid bilayer on the surface of the iron oxide NPs may act as a lipidic membrane, therefore improving the potential of iron oxide NPs as negative contrast agents for magnetic resonance image.

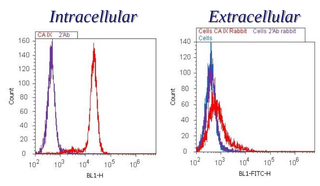


PRODUCTION OF MONOCLONAL AB ANTI-CA IX

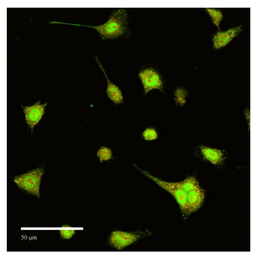
1 – Test the presence of CA IX in tumor cells



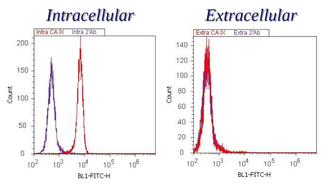
Confocal microscopy of SaOs-2 cells stained with TO-PRO 3 (red) and CA IX commercial antibody (green).



Flow cytometry of SaOs-2 cells show the presence of CA IX intracellular.

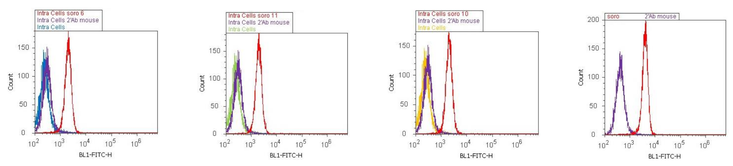


Confocal microscopy of MDA-MB 231 cells stained with TO-PRO 3 (red) and CA IX commercial antibody (green).



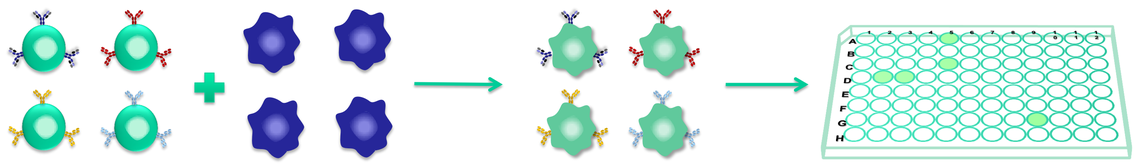
Flow cytometry of MDA-MB 231 cells show the presence of CA IX intracellular.

2 – Immunization

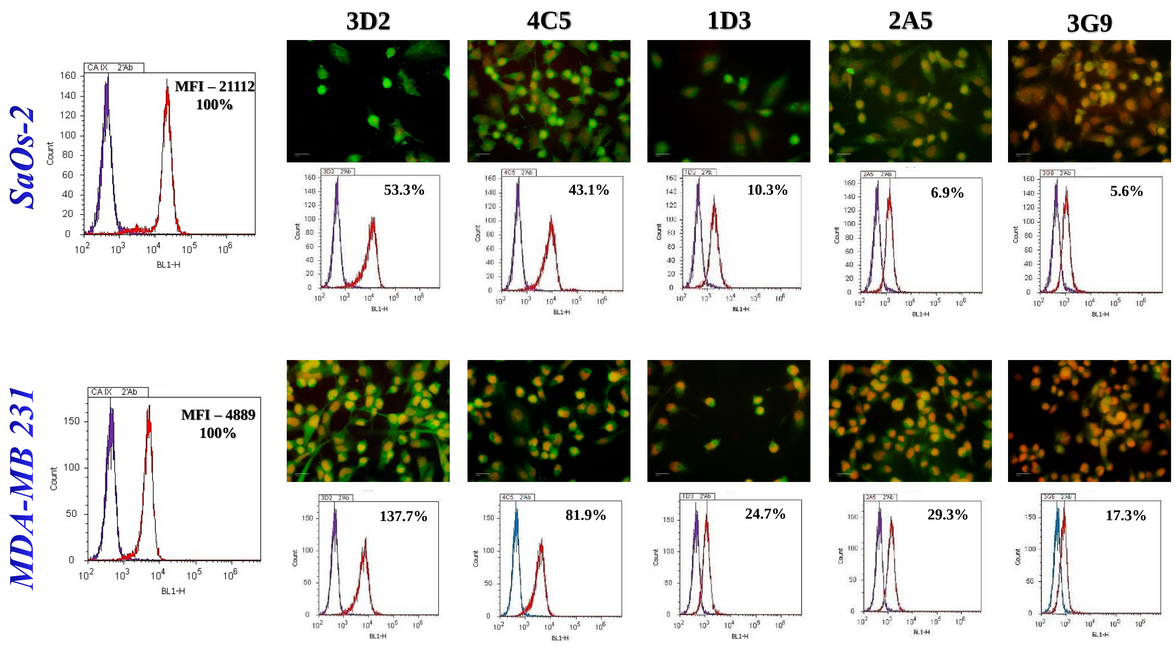


Flow cytometry of the serum of several immunized mouse showed the presence of antibodies against CaIX.

3 – Cell Fusion



4 – Screening



The present work represents the development of monoclonal antibodies against a transmembranar glycoprotein – Carbonic anhydrase IX (CAIX). This glycoprotein belong to a family of ubiquitous metallo-enzymes that act as catalyst of the reversible hydration of CO_2 to HCO_3^-. CAIX in particularly is over expressed in several tumor cells, mainly in solid tumors, invariably linked to with the hypoxic phenotype.

In a first step of the work, the presence of CAIX was tested in to tumor cell lines – SaOs-2 and MDA-MB 231. These cells were grown in normoxia, therefore the presence of CAIX was not identified in the membrane surface. However, when cell were permeabilized, the presence of CAIX was identified.

The second step of this work was the immunization of mouse with the antigen. The serum of the mouse was evaluated by flow cytometry, and the presence of antibodies anti-CA IX was confirmed. Spleen B cells were isolated from the mouse and fused with myeloma cells. The resulting hybridomas were tested as antibody producing cells by flow cytometry and immunofluorescence. The results show that the hybridomas 3D2 are producing antibodies with a high specificity for CAIX, although the protein is not presence in the cell surface.

Further work includes inducing hypoxia in both cells lines and confirm the overexpression of CAIX in this condition. Regarding the hybridomas, the selected ones will be subcloned and the resulting antibodies purified.

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6. Soares P, et al, Journal of Nanoparticle Research, submitted.

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CEDOC Chronic Diseases FCM NOVA

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