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Multimodal RM and Optic Imaging using ultramagnetic liposomes to monitor a cancer therapy

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Abstract – *Magnetic liposomes present an opportunity for the development of theranostic systems. Ultra-magnetic liposomes (UML) are highly loaded with ultra-small particles of iron oxide (USPIO) and can accumulate in a biological area thanks to a magnet. The incorporation of a fluorophore such as cyanine5.5 in the formulation of the UML forms a bimodal imaging agent (MRI and optic) to monitor the magnetic accumulation in the tumor. The addition of an antitumoral drug into the UML combined with the application of High Intensity Focused Ultrasounds (HIFU) in the tumor can be used for a specific release of the drug in the region of interest.*

Index terms - *Magnetic Resonance Imaging, Nano medicine, Optical Imaging*

I. INTRODUCTION

Nanotechnologies are booming and their association with therapeutic vectorization allows us to develop new injectable agents targeting a specific biological area to monitor a treatment. In this study, we design a thermo-sensitive ultra-magnetic liposome with bimodal imaging properties: optic and MRI, and with magnetic targeting capacities to reach implanted tumors in small animals. Liposomes can encapsulate an antitumoral drug to treat cancerous cells, decreasing the side effects of classical chemotherapies by the magnetic targeting. Dynamic MRI imaging methods enable the *in vivo* biodistribution evaluation of these theranostic agents to visualize and optimize the specific tumoral targeting. Fluorescent methods allow the accumulation observation in real time with the magnet.

II. MATERIALS AND METHODS

II.1. Synthesis of Ultra Small Particles of Iron Oxide (USPIO)

The nanoparticles of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) were synthesized according to the Massart's procedure [1]. They were citrated and suspended in an aqueous physiological buffer. The volume fraction and the average

magnetic diameter were determined by fitting the magnetization curve of the magnetic nanoparticles using the Langevin's law. The average core diameter was measured by Transmission Electron Microscopy.

II.2. Preparation of Ultra-Magnetic Liposomes (UML)

Mixtures of DPPC/ DSPC/ DSPE-PEG₂₀₀₀/ DSPE-PEG₂₀₀₀-Cy5.5 (85.5/ 9.5/ 5/ 0% mol [2] or 81.8/ 8.4/ 4.7/ 5%mol) were dissolved in diethyl ether and chloroform, and aqueous USPIO were added. To obtain a water-in-oil emulsion, a sonication at room temperature was performed during 20min. Then, organic solvent was quickly evaporated at 25°C with a rotavapor to form a gel phase. Liposomes were filtrated through a 0.45µm filter. The liposomes were separated from the non-encapsulated maghemite nanoparticles using a strong magnet. The average core diameter and the polydispersity of the liposomes were determined by TEM.

II.3. Cytotoxicity Assay

The cytotoxicity of USPIO and the various formulations of ultra-magnetic liposomes were analyzed *in vitro* by a mitochondrial assay using MTT. These assays were performed on BWGT3 hepatocyte, Raw macrophage and CT26 colon tumor cells. [3]

II.4. In vitro studies

Relaxivities r_1 and r_2 of USPIO and UML were measured at 4.7 and 7T (Bruker).

The magnetic targeting was tested *in vitro* in tubes with 1 or 2 magnets. This experiment was made in water or through a 2% agarose gel. The motion of UML was observed with a fluorescence camera (Biospace and Apogee).

II.5. In vivo studies

The *in vivo* studies are made on Balb-C mice.

The evaluation of the biodistribution and more precisely of the hepatic accumulation was performed after a 100µL IV injection of UML at 5mM of iron by Intragate and standard Dynamic Susceptibility Contrast Imaging methodology at high temporal resolution (30min post injection and sequentially during 1 week) at 7T.

The magnetic targeting in the tumor was validated *in vivo* on a CT26 murine model by MRI with a T_2^* weighting and by fluorescence imaging to observe the accumulation of the UML into the tumor.

III. RESULTS

The magnetic nanoparticles used in this study have an average magnetic diameter of 10nm and core diameter of 9.8nm with a polydispersity of 0.29. They are encapsulated in liposomes with a core diameter around 251nm with a polydispersity of 0.31. The r_2/r_1 doubles between USPIO alone and while they are encapsulated in liposome.

The figure 1 shows the accumulation of the fluorescent UML *in vitro* under the action of 2 magnets.

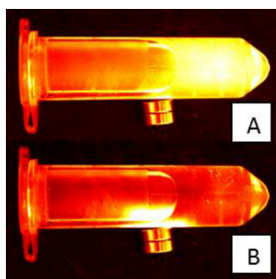


Figure 1: UML with 5% of Cy5.5 observed in water on fluorescence imaging 10s (A) and 10min (B) after the application of the magnets. Excitation filter: 670nm, Emission filter >700nm.

The biodistribution of the fluorescent UML are observed by MRI and by fluorescence imaging and, as shown in figure 2, they accumulate in the liver.

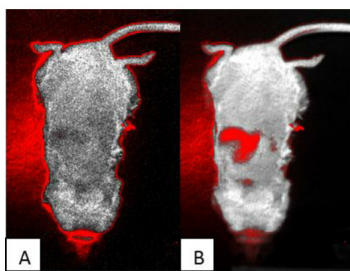


Figure 2: Fluorescence visualization of the accumulation of the fluorescent UML in the liver before (A) and 1h after the IV injection.

The figure 3 is a good example of magnetic targeting into a CT26 implanted tumor in a mouse. The T_2^* weighted MR image shows the apparition of a hyposignal in the tumor due to the accumulation of the UML. The number of pixels in the 25 percentile intensity histogram is calculated before and 30min after the injection. The ratio between these 2 numbers is almost doubled in the case of a magnetic targeting compared to a simple EPR accumulation.

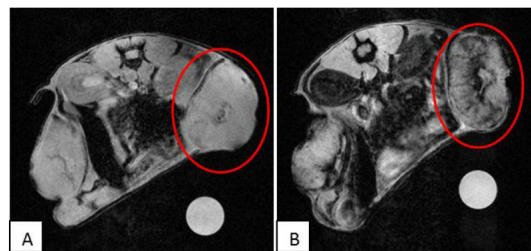


Figure 3: T_2^* weighted image of the implanted tumor before (A) and after (B) the injection of the UML and a 30min magnetic guidance.

IV. DISCUSSION – CONCLUSION

The relaxivity measurements reveal compatible values with the use of these liposomes as T_2 contrast agents in MRI. Their size, stability and non-toxicity assure their IV injectable capacities. The magnetic targeting of the UML up to the tumor allows an increase of their accumulation compared to a classical EPR effect. This accumulation is revealed by an increase hyposignal in T_2 weighting in MRI and an increase of the signal in fluorescence imaging. The use of high intensity focused ultrasounds (HIFU) will allow an active release of the drug in the tumor and a better penetration into the tumoral cells.

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