In vivo quantitative cellular MR imaging guided by in vitro relaxation parameter measurements of magnetic labeled cells

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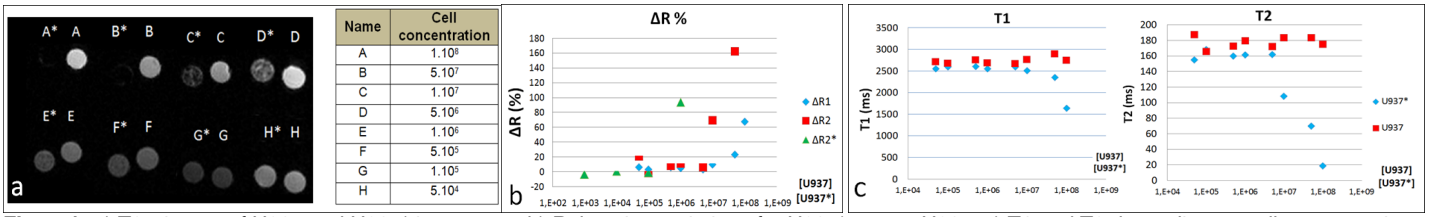
Introduction: This study is focused on the cellular MRI using ultra-small super-paramagnetic iron oxide (USPIO) nanoparticles which plays a key role to follow and monitor the efficiency of cellular therapy in vivo. Here, we aim to determine accurate MRI parameters in term of sensitivity to better visualize and quantify the fate of therapeutic cells (their volume distribution and their effects on tumor tissue) in a longitudinal study. In vitro experiments were conducted at different cell concentrations and a longitudinal in vivo study on a glioma mouse model was achieved.

Subject and Method ***Cell labeling and characterization:*** U937 macrophages labeled with Molday Ion RhodamineB (MIRB), a bi-functional super-paramagnetic and fluorescent nanoparticle (1), referred here as U937* were used. Cell labeling was characterized in vitro. U937 cells were incubated in a culture media including the MIRB nanoparticles at the concentration of 15 µg Fe/ml (2). Centrifugation at 600 rpm was applied to extract the internalized cell population without free nanoparticles. The cell viability and its proliferative capacity were studied with regular cell counts andMTT assays. Cell iron concentration was quantified by inductively coupled plasma spectroscopy. A follow-up of the phagocytosis of MIRB by U937 was assessed using confocal microscopy and flow cytometry. The incubation time was fixed to 20 hours. All the interactions of MIRB with U937 were compared to control samples, prepared in the same condition but without nanoparticle addition. ***Phantom preparation*****:** U937 or U937* cells in culture media were uniformly mixed to an agarose gel of 1% at a temperature in the range of [37°C - 40°C]. The mixture was cooled in 1.7mm diameter tube and quickly frozen to prevent cell sedimentation. Different cellular concentrations from 4.10^4 to 10^8 were prepared (Fig.1a). ***Animal model*****:** 5.10^5 GFP U87 cells were injected in the brain of nude mice (n=9). After 3 weeks of tumor growth, 5.10^5 U937* were injected at the tumor site (n=3). MRI was achieved every week in vivo to monitor the tumor growth as well as the spatial distribution of U937*. ***MRI:*** Quantitative T_2^*, T_2, and T_1 images were obtained for both in vitro and in vivo experiments**.** T_2^* maps were obtained using multi gradient echo sequence (TE/TR = 3.7/1500ms), T_2 maps using multi-slice/multi-echo (MSME) sequences and T_1 maps using RARE_vtr (TE=10ms, TR=4000, 2700, 1700, 1200, 800 and 400 ms). T_2 weighted images were performed using TurboRARE sequence (TE/TR = 10/2500ms). 7 slices of 600 □m thickness were acquired with a spatial resolution of 100 x100 μm^2 in plane. All MRI acquisitions lasted one hour. Data analysis and map reconstructions were achieved using Matlab software.

Results*:* The rate of cell proliferation and the percentage of viable cells were not affected by the MIRB internalization. In vitro, the relaxation parameter variations versus cell concentrations show different behaviors (Fig.1b). Indeed T_2 and T_1 parameters appear relevant for high labeled cell concentrations, upper than 1.10^7 cells/mL (Fig 1c). More specifically T_1 parameter appears the appropriate parameter to quantify labeled cells at very high concentration with a good sensitivity that can allow quantification. T_2^* MRI is a highly sensitive parameter with a unique efficiency to detect and quantify low labeled cell concentrations (Fig.1b). In vivo, U937* cells are detected with a loss of signal on T2 weighted images (Fig.2a). The attenuation of signal loss in days 9 and 12 is due to U937* cell division and spatial distribution leading to lowest U937* concentrations. The relaxation variations, measured in U937* ROIs versus contralateral ROIs, show similar tendency of MRI parameters than in vitro (Fig.2B). The relaxation rate variations ΔR2* is at the maximum until day 12 where it decreases for low U937* concentrations whereas ΔR1 and ΔR2 decrease in early days, for high U937* concentrations.

Discussion/conclusion: This study shows which MRI parameter is more suitable to track magnetically labeled cells and shows the ability to quantify labeled U937* cell concentration in a longitudinal study. As a first conclusion, T_2^* is suitable for low concentrations (1.10^5 to 5.10^6 cells/mL) whereas T_1 is appropriate for higher concentrations (5.10^6 to 1.10^8 cells/mL). In vitro studies were performed at different U937* concentrations with constant amount of intracellular nanoparticles. This is not the case for in vivo experiments because upon cell division, the amount of nanopaticles of each daughter cell is halved. For quantification of labeled cell concentration in vivo, an in vitro study on relaxation versus the amount of intracellular nanoparticles should be performed. MRI results validation by ex vivo fluorescent microscopy is under development as well as the use of other innovative therapeutic cells.



Name	Cell concentration
A	1.10^8
B	5.10^7
C	1.10^7
D	5.10^6
E	1.10^6
F	5.10^5
G	1.10^5
H	5.10^4

Figure1: a) T2w image of U937 and U937* in agarose. b) Relaxation variations for U937* versus U937. c) T1 and T2 depending on cell concentration.

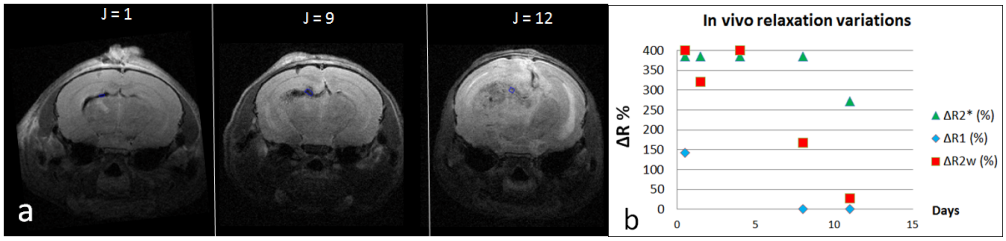


Figure2 : a)T2w images of the same mouse injected with U937* cells. b) Longitudinal evolution of relaxation variations between U937* ROIs and contralateral ROIs, ΔR maximum is limited at 400%.

(1) Biopal (Worcester, MA, USA) **(2)** Addicot et al., 2011, Contrast Media Mol. Imaging