Magnetic-Fluid-Loaded Liposomes (MFLs) for Selective Targeting and Treatment of Brain Tumors: example of combination of multi-scale approaches to validate *in vivo* magnetic targeting.

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1. INTRODUCTION

Hybrid devices based on the association of iron oxides with lipid nanoscale particles play an increasing role for targeted delivery of chemotherapeutics, mainly due to their biocompatibility and intrinsic efficacy as contrast agents for *in-vivo* Magnetic Resonance Imaging (MRI). In this study, we aimed at targeting human U87 glioblastoma, implanted into the striatum of mice, using magnetic-fluid-loaded liposomes (MFLs). MFLs targeting was achieved by applying a magnetic field gradient, from a magnet placed onto the head of the mice.

Challenge here was to find 200 nm diameter particles randomly dispersed into a complex tissue (glioblastoma). To avoid looking for a needle in a haystack, we selected MFLs-enriched regions on 70 µm sections, prior to TEM or EFTEM preparation.

2. RESULTATS

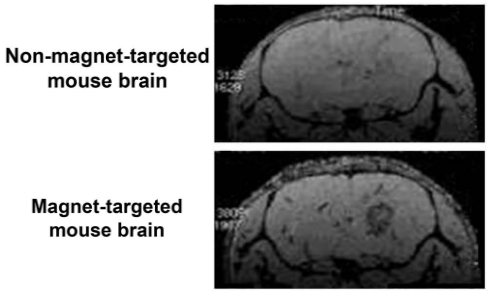
2.1 Experimental conditions

Prior to imaging, brain tumors were induced by implanted human U87 glioblastoma, into the right striatum of mice. Glioblastoma tumors were then allowed to grow for two weeks. MFLs were administered to mice by injection into the caudal vein. MFLs targeting was achieved by applying a 190-T/m magnetic field gradient, produced by an external 0.4-T magnet placed onto the head of the mice.

Four hours after MFLs injection, *in vivo* MRI experiments were performed on a 7 Tesla Avance III Biospec 70/20 USR (Bruker). Animals were then perfused by intracardiac injection of saline buffer followed by a fixative solution (1.5% GA, 1% PFA in 0.1M PB pH 7.3). Brains were collected and sliced into 70 µm sections with a vibratome, processed alternatively for light microscopy or Transmission Electron Microscopy (TEM), to perform cytological analysis (Zeiss 912omega) or Energy-Filtered TEM (EFTEM) for chemical mapping (JEOL 2100 with Gatan Gif Tridiem spectrometer).

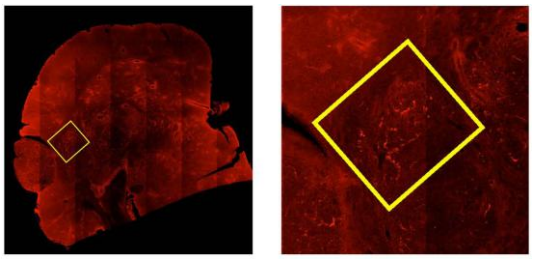
2.2 Magnetic Resonance Imaging:

The monitoring of MFLs targeting was achieved with T2*-weighted 3D gradient echo (GE T2*) sequences. This setting revealed the presence of magnetic fluid enclosed in MFLs as hypo-intense signals (lower). Indeed, at 4 h post-injection, the tumors of the magnet-exposed animals were seen markedly dark (lower) in comparison with the non-exposed ones (upper). MR



images demonstrated a significant accumulation of MFLs in glioblastoma under the influence of the magnetic field gradient.

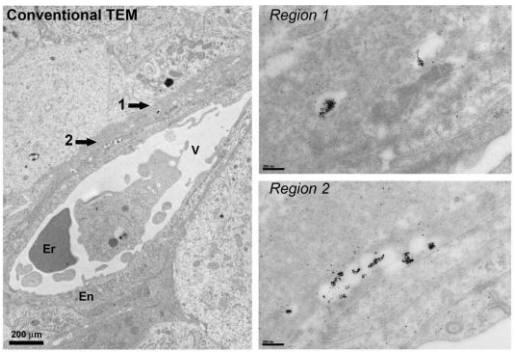
2.3 Localization of region of interest (ROI) for electron microscopy



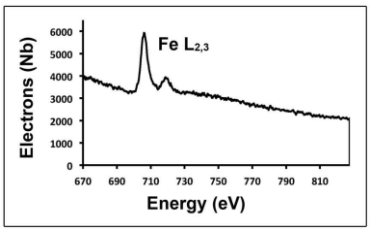
Fixed brains were serially sectioned (70 µm thickness) using a vibratome. Sections containing tumor tissue were collected and alternatively prepared for light microscopy or embedded in epoxy resin for TEM or EFTEM. As control, contralateral brain regions chosen in the striatum tissue were processed according to the same protocols.

ROI was targeted prior to ultramicrotomy: rhodamine labeling, inserted within lipid membranes of MFLs, was detected using confocal microscopy. Tumor areas presenting accumulation of MFLs were selected and the region of interest was trimmed on adjacent 70 µm thick sections previously processed for TEM or EFTEM (yellow box).

2.4 Cytological analysis and chemical analysis in Transmission Electron Microscopy



TEM was conventionally performed at 80kV on ROI previously selected through confocal microscopy in adjacent slices. TEM revealed the presence of clustered electron-dense nanoparticles in the extracellular matrix space and within endosomal-structures (right) in the cytoplasm of tumour cells and in cells lining the vascular lumen (left). Electron dense nanoparticles were observed only in glioblastoma tissue from mice injected with MFLs and exposed to the magnet. Contralateral regions of the same mice, used as control, did not present these electron-dense clusters. Scale bar: 200 µm. En: Endothelial cell, Er: Erythrocyte, V: Vessel.



EFTEM was then performed at 200kV on a set of adjacent brain slices, prepared without any contrasting agents. Parallel Electron energy-loss spectroscopy (PEELS), applied to the electron-dense clusters revealed values identified as $L_{2,3}$ edges (710-723 eV) of Iron. Electron spectroscopic iron mapping (ESI), confirmed their iron composition, allowing the identification of maghemite contained within MFLs *(H. Marie et al 2015)*.

3. CONCLUSION

The overall observations showed that MFLs were successfully delivered and concentrated into glioblastoma via the vasculature. They pass through the vascular endothelium as intact structures due to permeation and retention effect significantly enhanced by magnetic targeting before to be internalized by the tumor cells. Interestingly, the magnetic field gradient does not affect the amounts of MFLs recovered in the healthy part of the brains, which comparatively remains very low according to the different imaging methods implemented in this work.

The results in their whole revealed MFLs as potent tools for selective targeting of malignant brain tumors, especially promising for therapeutic issue as it can be expected that healthy brain tissue will be spared upon treatments by deleterious anticancer drugs carried by MFLs.

REFERENCE

Marie H., Lemaire L., Franconi F., Lajnef S., Frapart Y.M., Nicolas V., Frébourg G., Trichet M., Ménager C., Lesieur S. 2015. Superparamagnetic Liposomes for MRI Monitoring and External Magnetic Field-Induced Selective Targeting of Malignant Brain Tumors. Adv. Funct. Mater. 25, 1258–1269. DOI: 10.1002/adfm.201402289