Carboxy-silane coated iron oxide nanoparticles: a convenient platform for cellular and small animal imaging

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Purpose :

In this study, USPIO were stabilized with 3-(triethoxysilyl)propylsuccinic anhydride (TEPSA) by using a simple coating protocol that ensures the formation of a thin carboxylated layer of polysiloxane. The presence of carboxylic functions allows the covalent tag of the nano-platform in order to improve its performance. As a prototype, we combined a fluorescent compound, rhodamine, to the synthesized MRI platform. Such modification allowed us to follow directly these nanoparticles (NP) *in cellulo*, providing interesting information about their internalization pathway and cellular distribution upon mitosis. Finally, the performance of the nano-system as a potential probe for bimodal imaging was evaluated in a mouse model by studying NP distribution by magnetic resonance and optical imaging.

Methods:

The HeLa cell line was purchased from the NIH AIDS Reagent Program. The FIB364 cell line was derived from HeLa and stably expresses Fibrillarin-GFP. The H2B-GFP HeLa cell line was a gift from Dr. B. Farhenkrog (ULB, Université libre de Bruxelles, Belgium). U2OS cells stably expressing the GFP-G3BP1 construct were made available by Prof. P. Anderson (Brigham and Women's Hospital, Boston, USA). Cells were cultured in DMEM supplemented with FBS (10%) from PAA and Pen/Strep (50 µg/ml) from Gibco under 5% CO_2 in a humidified incubator. MR imaging was performed on a 9.4 T Biospec (94/20, Bruker, Ettlingen, Germany). One-millimeter thick image slices were collected using a RARE sequence. The parameters for localized USPIO-related T_2 signal decrease were: TR/TE=2500-3634.1/40.21 ms, RARE factor=8, MTX=384x256, FOV=35x25 mm, 2-4 averages, TA=3min53s. USPIO-enhanced MR images were recorded in healthy mice derived from the BalbC strain (Charles River, L'Abresle, France) and in mice bearing tumor (with the model developed on the DBA/2 mouse by the team of Prof. Muriel Moser at the Immunobiology Laboratory, ULB-Gosselies). Optical imaging was performed using a PhotonImager (Biospace Lab, France).

Results and discussion:

USPIO prepared by co-precipitation of iron salts in PEG were stabilized with an organosilane (TEPSA) exhibiting carboxylic functions for the grafting of a rhodamine fluorescent probe (Rh). These TEPSA-Rh-PEG NP were characterized by photon correlation spectroscopy, electron microscopy and relaxometry.

Cellular study: No significant effect on cell viability was detected after the incubation of HeLa cells with 50 µg Fe/ml of NP over a period of 6 days. The intracellular distribution of NP in both HeLa and U2OS cells was investigated. For co-localization purposes, we used HeLa cells that stably expressed the nucleolar protein fibrillarin fused to the green fluorescent protein (GFP) and U2OS cells stably transfected with a GFP-G3BP1 construct that decorates cytoplasmic stress granules (SGs). In both HeLa and U2OS cells, NP appeared concentrated in small cytoplasmic *foci*, which were excluded from the nucleus and nucleolus, and did not co-localize with SGs. This localization, which is highly reminiscent of perinuclear membrane-bound organelles, suggested that TEPSA-Rh-PEG NP are captured by endocytosis, ending up in lysosomes. We established the extent to which the nanoparticles are transmitted between mother and daughter cells following mitosis. To this end, we performed live-cell imaging and we precisely quantitated the amounts of NP partitioned between the progeny. We concluded that following mitosis, the NP redistribute rather homogeneously with a calculated average ratio over 50 mitotic events of 1.1 between the daughter cells. Further, and in agreement with our observation that NP are non-toxic to cultured cells, we did not detect any influence of the presence of NP on cell division.

Small animal imaging: For *in vivo* imaging experiments, the TEPSA-Rh-PEG NP were injected intravenously *via* the tail vein in a tumor-bearing DBA/2 mouse and in healthy mice derived from the BalbC strain. MR signal darkening was observed in the liver, this negative contrast in the liver persisted for several days (until 3 days *post*-injection), in accordance with the role of USPIO metabolization played by the liver. MR imaging was also focused on the urinary bladder, which showed signal darkening over an observation time of 2 hours, suggesting that some injected USPIO are small enough (~ 10 nm diameter) to undergo renal filtration. Signal darkening was observed in the blood circulation, as illustrated in coronal images by the hypo-contrasted *vena cava* 15 min after injection of the nanoparticles in a BalbC strain-derived mouse. In the DBA/2 mouse, as an illustrative main blood vessel, the *aorta* appears black-colored within the direct post-injection observation period (2 hours).

Using *in vivo* optical imaging, results suggested that the amount of USPIO in specific tissues was sufficient for its fluorescent signal to be detected in the whole tumor-bearing mouse.

Conclusions:

In this study, we demonstrated that TEPSA-Rh-PEG NP are potential bimodal contrasting agents. The persistence of NP in the intravascular compartment allowed for the depiction of tumor blood vessels in the subcutaneously-implanted *mastocytoma*. With adequate PEG-related stealthiness, the USPIO could thus be an interesting MRI tool for the analysis of the intravascular compartment in tumor or brain perfusion studies that would benefit from their blood pool and steady-state susceptibility contrast agent properties. Finally, *in cellulo* studies provided interesting information about the internalization pathways and cellular distribution upon mitosis of the here-presented USPIO. We found that cultured human cells naturally uptake these NP at the optimal concentration of 50 µg/ml Fe; that the NP accumulate in lysosomes indicating that they are entering cells by endocytosis; and that the NP are equally partitioned between daughter cells following mitosis. These nano-systems are non-toxic to mouse and human cultured cells as they do not impact the average time for mitosis completion or cell viability.