In vivo visualization of cells marked with ultra-small Gd2O3 nanoparticles, using a 1.5 T clinical system and the chick embryo model

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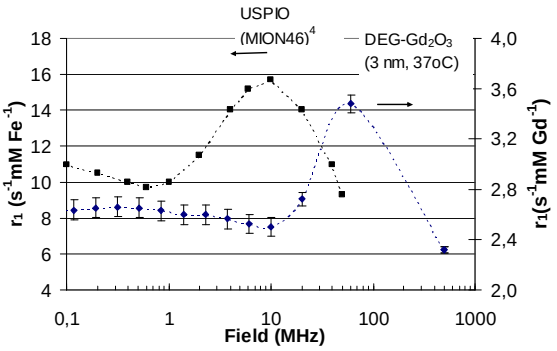
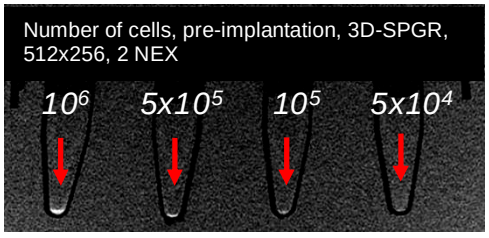
Background: the visualization of small clusters of cancer cells in a vascularised *in vivo* model is crucial in the development of efficient methods to study and follow-up tumour and metastasis growth. The chick embryo chorioallantoic membrane assay has been used as a convenient, non-rodent model to study angiogenic response with cancer cells implanted *in vivo.[1]* In the context of MRI and contrast agents research, this model could represent a versatile tool to rapidly visualize small clusters of marked cells in a vascularised environment. This could allow for follow-up studies with clinical imaging systems available in most research hospitals. At the moment, cell staining and tracking with iron oxide nanoparticles (USPIO) is widely established: susceptibility artefacts resulting from strong spin-spin interactions cause hypo-intense contrast in MR images. Incubation of cells with USPIO is associated with limitations: T_2/T_2* weighting sequences are used, that in general provide images of lower resolution than T_1-weighted ones. Hypointense signal and image artefacts generated by the presence of USPIOs in clusters of cells does not always allow an effective differentiation between the cells and the background. In order to obtain positive contrast from transplanted cells, we have developed a labelling method to incorporate ultra-small Gd_2O_3 nanoparticles into cancer cells. Glioblastoma multiforme is one of the most lethal forms of neurological malignancies, and ultra-small nanoparticulare gadolinium-containing compounds could become a promising tool for brain cancer Gd internal radiation neutron capture therapy (Gd-NCT). The contrast agent could therefore be used for both diagnostic and therapy.

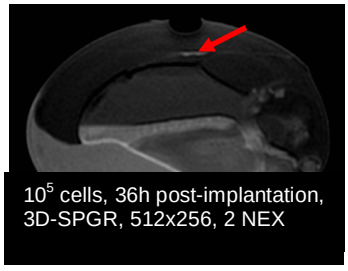
Materials and methods: *Gd_2O_3 nanoparticles* were synthesized in a high boiling point alcohol.*[2]* The colloids are made of more than 200 Gd ions arranged in a cubic closed-packed structure. They were adequately filtered, dialyzed and analyzed with transmission electron microscopy and dynamic light scattering (Malvern Nanosizer 173) to establish their size distribution. Aqueous suspensions of dialyzed nanoparticles were analyzed for T_1 and T_2 at 60 MHz with a dedicated relaxometer (Bruker Minispec) and at varying external magnetic field by NMRD (nuclear magnetic relaxation dispersion, Stelar). The particle suspensions were further stabilized with PEG-silane*[3]* and the resulting compound was used to incubate glioma cells. *In-vitro cell studies:* glioma cells were grown to confluence and incubated with large concentrations of contrast agents (0.75-1 mMol Gd). Uptake and retention studies were performed: a rapid oil filtration method was used to separate Gd internalized in glioma cells from the fraction of Gd corresponding to loosely attached nanoparticles and contaminating incubation medium. Then, the viability of Gl-261 glioma cells incubated with US-Gd_2O_3 was measured after 4h, 8h, 24h and 48h. Amounts of Gd in aqueous suspensions and in cells were measured with ICP-MS. *MRI visualization of marked glioma cells in eggs:* Cells were harvested, counted, visualized with MRI in tubes, and then implanted (100 000 cells per egg) in 11 days old chicken embryos, at the surface of the chorioallantoic membrane (CAM). The eggs were imaged after 36, 48 and 60 hours post-injection with a 1.5 T GE Signa clinical system, using a wrist coil and 3D-SPGR sequences providing a resolution down to 250 µm. Embryos were sacrificed after 7 days of implantation, and tumours were extracted.

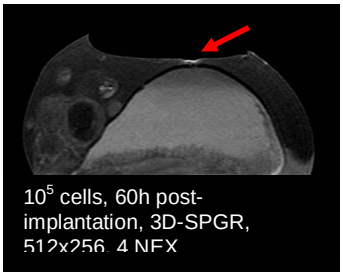
Results and discussion: *Relaxometric studies:* The ratios between transversal and longitudinal relaxivities (r_2/r_1) in suspensions of US-Gd_2O_3 nanoparticles (hydrodynamic diameter of about 3 nm) are 1.3-1.4(@60MHz), confirming the strong potential of this contrast agent for T_1-weighted "positively" contrasted imaging. Longitudinal relaxivity NMRD profiles (r_1) revealed a peak that is shifted towards higher magnetic fields compared to reported profiles for USPIOs.*[4]* This is a very promising indication that US-Gd_2O_3 could provide a positive signal even at magnetic fields of 3T. *In-vitro cell studies:* no inflexion was found on the growth curves, and cell viability studies did not reveal evidences of toxicity after 48 hours of incubation with the contrast agent. The labelling efficiency was about 2-10 pg Gd/cell. Only a small fraction (1-2%) of gadolinium is loosely attached at the surface of cells. *In vivo and in vitro imaging:* clusters of cells down to 50 000 could be distinguished *in vitro*, and 100 000 positively contrasted cells were successfully visualized *in vivo* using 3D-SPGR T_1 weighted sequences. After 7 days, vascularised xenograft tumours could be extracted in a large fraction of implanted eggs.



Conclusions: Efficient labelling of cancer glioma cells is possible with US-Gd_2O_3 nanoparticles. Implantation of marked cells in the chick embryo provide a convenient vascularised model, readily available, less expensive than rodents, and allowing for tumour development studies involving new MRI contrast agents and the use of widely available MRI clinical systems. This model could also be used for pre-clinical Gd-NCT internal radiation therapy studies.







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