Quantification of Iron Oxide Nanoparticles in a large animal model using MRI

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Purpose: Nanostructures are poised to play a crucial role in medicine by serving both as carriers for drugs and imaging agents that will selectively bind to targets on diseased cells and tissues. In this context, it is very important to develop tools to evaluate and detect these therapeutic agents for potential use in pre-clinical development and in clinical trials. The EU-funded MULTIFUN consortium, including our group, develops multifunctionalised superparamagnetic iron oxide nanoparticles (SPIONs) combining diagnostic and therapeutic features against breast and pancreatic cancer. Although originally developed as liver-specific contrast agents, SPIONs are widely used as drug carriers/imaging agents due to their strong magnetic properties. Hence, the distribution of SPIONs in the body (biodistribution) can be determined using Magnetic Resonance Imaging (MRI). The aims of this study are, to establish an MRI protocol for the quantification of SPIONs in ex-vivo pig organs and to analyse the levels of SPIONs in kidneys.

Methodology: Fifteen (OD15) mm and 12 mm (MF66) magnetite (Fe3O4) nanoparticles were investigated. The synthesis of OD15 was done via an organic route, while MF66 nanoparticles were produced in aqueous solvents. A range of phantoms tubes were prepared with different concentrations of each SPION (0.015 to 0.00469 mg/ml) in 1 % agarose to construct MRI calibration curves (R_2* versus concentration) which allows a quantification of each SPION in kidneys (Figure 1). The phantoms were scanned in a Philips 3T Achieva system with a 32 channel head coil using a T_2* sequence (multi-echo GRE), 32 echoes with TE=1.9+n(3.3) ms, FA=18°, 1 slice, resolution=0.5x0.5x2 mm^3, NSA=5, 9 min 12 s). To assess the biodistribution of our SPION (4), female landrace pigs (20–25 kg) were sedated intra-muscularly (i.m.) with ketamine (14 mg/kg) and xylazine (2.7 mg/kg) i.m. A cannula was inserted into an ear vein and the animal was anesthetized with a bolus, followed by a continuous intravenous (i.v.) infusion of sodium pentobarbital (induction 30 mg/kg; maintenance 6 mg/kg/h i.v.). Both SPIONs (0.5 or 2 mg/kg) or saline (control) were injected through the femoral vein as a bolus (n = 3 or 4, with 2 controls i.e. no SPIONs injected). After 5 hours, the animals were euthanised and the organs harvested and kept in a 10 % formalin solution. The kidneys were scanned with the same protocol except with NSA=20, resolution=0.8x1.2x2 mm^3, 4 min 30 s. The relaxivity of SPION was evaluated on a slice covering the entire organ. T_2* maps were constructed using MRmap software. For each kidney (in the cortex), T_2* was obtained by averaging the T_2* values of 20 regions of interest (ROI) using ImageJ software (Figure 2). SPION concentrations were determined from the calibration curves by computing the difference in R_2* values between controls and injected animals.

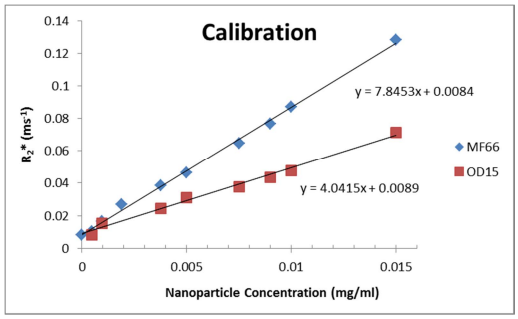
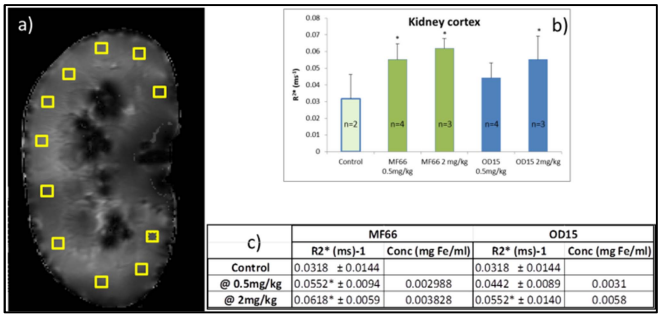


Figure 1



c)

	MF66		OD15	
	R2* (ms)-1	Conc (mg Fe/ml)	R2* (ms)-1	Conc (mg Fe/ml)
Control	0.0318 ± 0.0144		0.0318 ± 0.0144	
@ 0.5mg/kg	0.0552* ± 0.0094	0.002988	0.0442 ± 0.0089	0.0031
@ 2mg/kg	0.0618* ± 0.0059	0.003828	0.0552* ± 0.0140	0.0058

Figure 2

Figure 1. Calibration curves for the 2 types of nanoparticles.
Figure 2. a) Example of ROIs placed in the kidney cortex. b) R_2* values for SPION in kidney groups. c) Quantity of SPION in kidney groups based on the calibration curves. t-test, $p<0.05$ versus control.

Results: The R_2* ($1/T_2$*) values increased linearly as the concentration of SPION increases for the 2 types of nanoparticles (Figure 1). Depending on the relaxivity of each SPION, we obtained different equations from linear fits to the calibration data, which were subsequently used to calculate the concentration of MF66 and OD15 in the ex-vivo kidneys. MF66 showed enhanced relaxivity compared to OD15.
These iron based NPs have superparamagnetic properties and are known to behave as T_2/T_2* contrast agents, i.e., they produced darker images where they have accumulated (Figure 2a). At the 2 doses and for the 2 SPIONs, we observed an increase of R_2* values in the kidneys, revealing an accumulation of SPION particularly in the kidney cortex of all groups. There were significant differences between control group and both MF66 groups, with only a significant difference found between the control group and OD15 at 2 mg/kg injected concentration (Figure 2b). The table in figure 2c shows the amount of iron in kidneys corresponding to R_2* values, based on the calibration curves.
Discussion and conclusions: Nanomedicine is likely to represent a breakthrough in cancer patient treatment. It will personalize therapy and reduce side effects. The use of SPIONs as drug carriers/contrast agents will also facilitate the tracking of the therapeutic agents since SPIONs can be detected by non-invasive MRI due to their superparamagnetic properties. This study showed that it is possible to evaluate the quantity of SPION in ex-vivo pig organs (e.g. kidney). The values are not the total quantity of iron in the organ but represent an average value for each organ. This protocol can, with further development, potentially be used in cancer patients to visualise accumulation of SPION in the tumour. In fact, our nanoparticles are designed to recognise pancreatic and breast cancer cells through the use of specific antibodies, and to destroy them via 2 anti-tumour drugs (doxorubicin + Nucant speudopeptide) and by hyperthermia (wherein an externally-applied alternating magnetic field is used to infer kinetic energy to the SPIONs, thereby producing the appropriate heat capable of targeted cell destruction). By knowing the quantity of SPION in the tumour, doctors will be able to evaluate precisely the necessity or otherwise of the need to administer extra-dose(s) of functionalised SPION. Simply, if the quantity of SPION in the tumour is high enough to continue hyperthermia, there is no need of new administration. This study is a proof of concept of quantification of SPION in kidneys which can be translated to cancer patient treatment.