T_1 and T_2 Mapping of Superparamagnetic Iron Oxide Nanoparticles for the Detection of Breast and Pancreatic Cancer Cells

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Purpose

Limitations of current cancer therapies are nonspecific delivery and poor biodistribution of drugs as well as the lack of an effective modality for tracking delivery and monitoring treatment response. The aim of this project is therefore to develop and validate multifunctionalised magnetic nanoparticles (MF-MNP) to selectively target and monitor delivery and treatment response of MNPs by MRI. MNPs can be used as contrast agents and magnetic heating inductors and can be functionalised with targeting ligands to increase their affinity towards cancer[1]. Here we sought to investigate imaging properties of MNPs and validate these in breast and pancreatic cancer cell lines.

Methods

We assessed the MNPs supplied by the Multifun consortium (table 1) on their MR imaging properties and investigated their effects on various cancer cell lines. A water phantom was used to investigate the longitudinal and transversal relaxation rates of various MNP alone and 24 hours post incubation with human pancreatic carcinoma cells (i.e. PANC-1). We used a 2D multi-gradient-echo sequence (echoes = 5, slice thickness = 3 mm, TE = 1.7 ms, TR = 11 ms, FA = 25°) to measure T_2^* and a 2D multi spin-echo sequence to measure T_2. T_1 was determined using a sequence that employs two non-selective inversion pulses with inversion times ranging from 20 ms to 2000 ms, followed by eight segmented readouts for eight individual images[2] (fig 1). All experiments were performed on a clinical 3T Achieva scanner (Philips Healthcare, Best, NL).

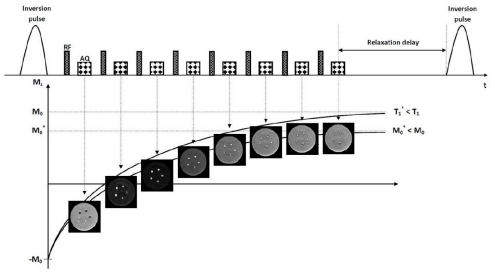

Figure 1: T_1 mapping Look-Locker pulse sequence scheme.

Results

The 15 nm DMSA-coated magnetic nanoparticle OD15 shows excellent r_1, r_2 and r_2^* values with no toxicological effects making it a promising contrast agent prototype for T_1, T_2 and T_2^* imaging (tab 1). Incubating human breast carcinoma (MCF-7) cells with OD15 indicated saturation after 24h. An increase in concentration of MNP resulted in more particles being bound to the cell. Electronmicroscopy (TEM) images confirmed that our MNPs are taken up by MCF-7 and MDA-MB-231 cells (fig 2a and b). The results from our viability assay showed that the cytoskeleton of the cells is not altered by the MNPs (fig 2d). We furthermore demonstrated normal morphology and distribution of interphase microtubules and no alterations in mitotic spindle organisation and chromosomes distribution (fig 2e and f). We compared free MNPs with MNPs incubated in PANC-1 cells regarding their R_2 and R_2^* behaviour. Cell-bound MNPs show a considerably stronger increase in the relaxation rate R_2^* compared to free MNPs (fig 3). Effects on R_2 are less pronounced in cell-bound MNPs than in free MNPs.

Name	Particle size TEM [nm]	Hydrodynamic size [nm]	Nature of coating	r_1 $[mM^{-1}*s^{-1}]$	r_2 $[mM^{-1}*s^{-1}]$	r_2^* $[mM^{-1}*s^{-1}]$
ADNH	6	150	Aminodextran	8.0	289.9	929.2
ASi	8.5	60	Aminosilane	11.1	118.0	272.0
OD10	10	50	DMSA	13.1	160.6	208.4
OD15	15	45	DMSA	13.1	130.0	318.6
MF66	11.7	85	DMSA	2.4	242.5	390.1
MF71	—	203	CM-dextran	1.4	110.0	287.8
MF74	—	90	DMSA	0.9	98.8	264.0
F1706	11.7	159	Dextran T40	3.1	145.1	289.2

Table 1: Promising SPIO nanoparticles.

Discussion

The DMSA-coated MNP OD15 shows good longitudinal and transversal relaxivities making it a promising candidate for MR imaging with T_1 and T_2-weighted sequences and for future multifunctionalisation experiment. It has been reported that r_1 and r_2 substantially decrease when the MNPs are internalised by cells as demonstrated in figure 3. This is because intracellular compartmentalisation restricts water diffusion and/or particle diffusion and renders MNPs detection more sensitive. Future aims will be the multifunctionalisation of OD15 to increase its affinity to specific carcinoma cells and to load the particle with anti-cancer drugs to also achieve a combined diagnostic and therapeutic effect.

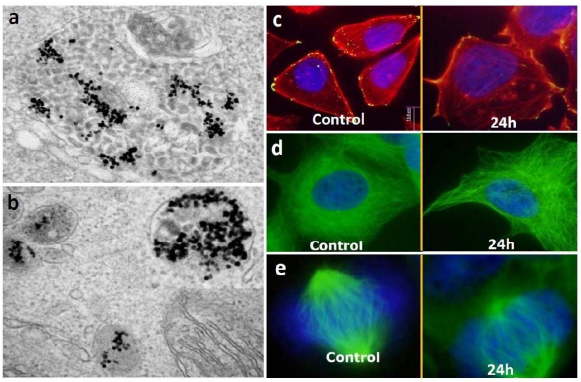

Figure 2: TEM images of a) MCF-7 cells and b) MDA-MB-231 cells incubated with OD15 for 24h. c) Indirect immunofluorescence for vinculin (green) and staining for F-actin (red). d) Immunofluorescence labelling of α-tubulin (green). e) Immunofluorescence labelling of α-tubulin (green) during cell division (mitotic spindle).

Conclusion

We demonstrate that the investigated nanoparticles have good MR imaging properties and can be used to label carcinoma cell lines. The investigated MNPs show promising imaging and labelling properties without affecting viability of the cells and thus warrant *in-vivo* investigation in nude tumour bearing mice (BT474, MDA-MB-231, BxPc-3 PANC-1) to evaluate biodistribution, specificity and suitability for in vivo imaging and therapy.

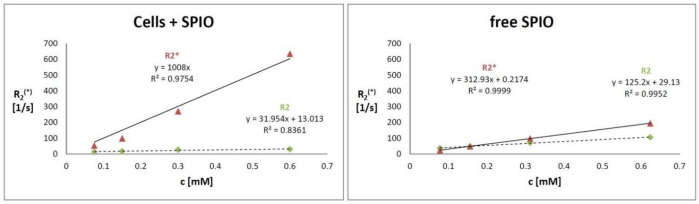

Figure 3: Comparison of R_2 and R_2^* of free and cell-bound SPIO.

References

[1] Peng, X.-H., et al., 2008. *Int J of Nanomed*, 3,pp.311-21
[2] Makowski, M.R., et al., 2011. *Nature Med*, 17,pp.383-88.