Investigation of a carcinoma-specific SPIO-antibody conjugate for MR visualisation of epithelial tumours

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Introduction:

Superparamagnetic iron oxide nanoparticles (SPIO) have a recognised potential as molecular MR contrast enhancement agents (1). It is possible to attach SPIO particles to tumour-specific antibodies, thus potentially improving the imaging and early detection of primary and metastatic disease. In the past, attempts have been made using antibodies recognising carcinoembyonic antigen (CEA) (2), human colorectal carcinoma (3) and human small-cell lung carcinoma (4).

We have covalently linked the 50nm dextran-coated Nanomag-D-SPIO (Micromod), via aldehyde linkage chemistry, to the genetically engineered humanised high affinity single chain variable fragment (scFv) "Sm3E". This chain recognises CEA, a glycoprotein associated with epithelial cancers. Using immufluorescent confocal analysis we have shown that the Sm3E-SPIO complex retains and shows specific binding to CEA-expressing LS174T cells (Fig 1,4). Here we present a pilot study of the MR properties of the Sm3E-SPIO complex and an *in-vitro* tumour cell study using the complex, confirming its specificity to the target cells, but also providing evidence of the tumour-specific MR contrast enhancement.

Methods:

Preparation of Sm3E-SPIO complex: The SPIO nanoparticles were oxidised using sodium periodate to allow aldehyde linkage to NH_3 groups on the scFv "Sm3E" and the aldehyde linkage was stabilised by the addition of sodium brohydride. This was followed by size exclusion purification to remove unbound Sm3E and sterile filtration. The final complex contains 40% iron.

Sm3E-SPIO tumour targeting *in-vitro* study: The CEA-positive colon cancer cell line LS174T and the CEA-negative melanoma cell line A375M were used. Three flasks of each line were set up and cells were incubated overnight in one of the following: 1) RPMI medium (Gibco) alone, 2) RPMI + SPIO 100ug/mL, 3) RPMI + Sm3E-SPIO 100ug/mL. The six samples were washed three times by centrifugation in PBS to remove any unbound SPIO or Sm3E SPIO and were fixed in 1% PFA. 1e+6 cells from each group were pelleted in one of six corresponding 250ul eppendorf tubes. The tubes were placed, with the pellets aligned, on a specially designed rack inside a 50mL falcon tube containing 2.5gL $CuSO_4$ (Fig 2) and were scanned along the long axis of the eppendorfs in a 2.35T horizontal bore SMIS system (3cm coil, 128x128 matrix, 1mm slice with 2 averages, FOV 40mm, TR=1s at TE=28,30,32,34,38,44,48,52,58,64,70,80). T2 values were derived from a ROI at the centre of the tumour cell pellet.

Concentration Study: Four dilutions of Sm3E-SPIO (0, 7, 37.5, 75 ug/mL) were prepared in PBS. These were placed in four 250uL eppendorfs and were scanned using a 3cm coil (128x128 matrix, 2mm slice along the eppendorf short axis, 2 averages, FOV 40mm, TR=1s, TE=28,30,32,36,38,44,54,64,74, 94,120,200,500). T2 values were calculated for each concentration.

Results:

Fig 2 displays the phantom designed for and used in the presented studies. Fig 3 demonstrates the decrease in the T2 values of the solution for increasing concentrations of the Sm3E-SPIO complex. Compared to the control concentration of 0 ug/mL (PBS only, no complex), there is an approximate 50% decrease in T2 at 7 ug/mL and an 80% decrease at 37.5 ug/mL). The iron concentration in these samples was 2.8ug/mL and 15ug/mL respectively. The complex shows specific binding to the CEA positive cell line LS174T and allows for visualisation of the cells using MRI (Fig 4,5). The cell pellet of the LS174T tumour showed at least a 40% decrease in T2 against all control measures (no SPIO, SPIO without Sm3E, SPIO-Sm3E on the CEA-negative tumour cell A375M), providing evidence of the specificity and potential of this complex.

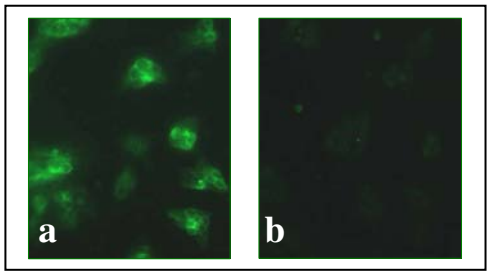

Figure 1: Confocal images using anti-dextran antibody (green) showing (a) binding of Sm3E-SPIO and (b) negligible binding of unconjugated SPIO, to LS174T cells.

Figure 2: Eppendorf rack
a) Side view of the rack with cell pellets visible (arrow). (b) The rack was scanned using a rat probe and a 3cm coil.

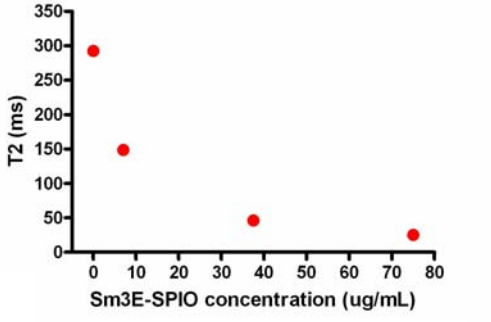

Figure 3: T2 values for increasing concentrations of Sm3E-SPIO

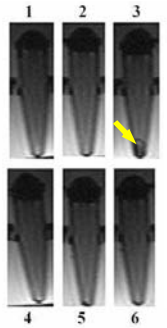

Figure 4: Tumour cell pellet MRI
1. LS174T + medium
2. LS174T + SPIO
3. LS174T + Sm3E-SPIO
4. A375M + medium
5. A375M + SPIO
6. A375M + Sm3E-SPIO

There is loss of signal in pellet no3 (arrow). TR=2s, TE=30ms

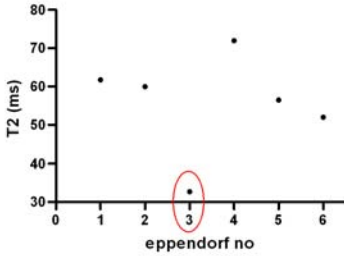

Figure 5: T2 value of tumour cell pellets

The eppendorf numbers correspond to the same cell pellets as in Fig 3.

Conclusions

We present a pilot study investigating the MR properties of the Sm3E-SPIO complex we have developed, together with evidence of the specificity of the complex for the LS174T tumour cells as visualised with MRI. In the future we plan to use this complex for non-invasive MR-based identification of primary and metastatic tumours in an animal model. Furthermore, we believe that the presented phantom design will prove an invaluable tool for our future work regarding MR contrast enhancement quantification and maximisation in tumour models and elsewhere.

References: 1.Bulte JW *et al.* NMR Biomed. **17**(7), 484-499 (2004) 2. Tiefenauer LX *et al.* Magn Reson. Imaging. **14**(4), 391-402 (1996) 3. Toma A *et al.* Br. J. Cancer. **93**(1), 131-136 (2005) 4. Remsen LG *et al.* AJNR Am. J. Neuroradiol. **17**(3), 411-418 (1996)