Novel receptor-targeted nanoparticles for MR imaging and specific delivery of gene therapy

P. Kyrtatos*[1], M. Writer*[2], A. N. Price[1], S. Hart[3], and M. F. Lythgoe[1,4]

[1]Centre for Advanced Biomedical Imaging, Institute of Child Health and Department of Medicine, University College London, London, United Kingdom, [2]Wolfson Centre for Gene Therapy of Childhood Disease, Institute of Child Health, UCL, [3]Molecular Immunology, Institute of Child Health, UCL, [4]RCS Unit of Biophysics, Institute of Child Health, UCL. *equal contribution to this work

Introduction:

Multimodal nanoparticles offer a promising application as MRI contrast agents with therapeutic capabilities (1). Our group is developing a novel gene therapy vector, which utilises an anti-cancer targeting peptide for specific transfection of tumour cells with therapeutic genes. In addition, a Gd3+ moiety has been incorporated for non-invasive real-time monitoring of the delivery. Here we present a pilot *in-vitro* tumour cell study investigating the MRI properties of the vector, confirming its potential for both specificity of delivery of DNA to the target cells for gene therapy and also providing evidence of the MR contrast enhancement in targeted cells.

Methods:

***In vitro* transfection**

Nanoparticles bearing the pCILuc plasmid containing a luciferase reporter gene were prepared both with and without the gadolinium label. For analysis of targeting ability, Gd-labelled nanoparticles were prepared using either a targeting peptide proven to increase nanoparticle uptake to U87-MG human glioblastoma cells (data not shown) or a random scrambled non-targeting peptide. For transfection, the nanoparticles were added in Optimem medium (Invitrogen) to the cells, spun down and incubated for 4 hours at 37°C. Unbound complexes were removed and the medium replaced, incubating the cells for a further 24 hours. Cells were lysed and reporter gene expression was analysed using a luciferase assay (Promega), measuring luminescence as relative light units (RLU) using a microplate reader.

MRI *in-vitro* study: Three plates of of 10^6 U87-MG cells were set up and incubated for four hours in one of the following: 1) Optimem alone, 2) Optimem + unlabelled nanoparticles, 3) Optimem + Gd-labelled nanoparticles. The three samples were washed in PBS to remove any unbound nanoparticles, detached using trypsin and pelleted. The cells from each group were resuspended in PBS, transferred to 250uL eppendorf tubes and repelleted. The tubes were placed, with the pellets aligned, on a rack inside a 50mL falcon tube containing 2.5g/L $CuSO_4$ (Fig 2) and were scanned along the long axis of the eppendorfs in a 9.4T horizontal bore Varian VNMRS system using a 2d spin-echo sequence (512 x 256 matrix, 0.5mm slice with 4 averages, FOV 60x30mm, TE=10.9ms at TR=0.25, 0.5 0,75, 1, 1.2 1.5, 2, 3, 5 seconds). T1 maps and values were derived from a ROI at the centre of each cell pellet using the ImageJ (v1.36b, NIH) MRI analysis plugin.

Results:

Fig 1 demonstrates that the incorporation of gadolinium into the nanoparticles does not significantly decrease the efficiency of transfection. Furthermore, exchanging a random scrambled sequence for a targeting peptide increases trasfection efficiency (Fig 2). Fig 2 displays the phantom used in the presented studies. The complex exhibits positive contrast in a T1-weighted image and allows for visualisation of the cells using MRI (Figs 4,5). The pellet of cells with the Gd-nanoparticle showed a 25% decrease in T1 and corresponding increase in signal intensity compared to unlabelled nanoparticle, providing evidence of the potential of this complex as an MRI contrast agent. Therefore, internalised Gd-nanoparticles exhibit positive contrast. It was also noted that free Gd-labelled nanoparticles did not exhibit positive contrast at the concentrations expected in the cells (not shown), which may indicate enhancement of MR contrast following internalisation.

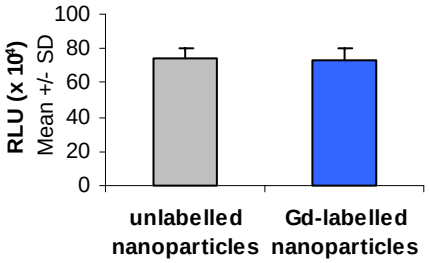

Figure 1: Tranfection efficiency of Gd-labelled versus unlabelled nanoparticles (luciferase assay)

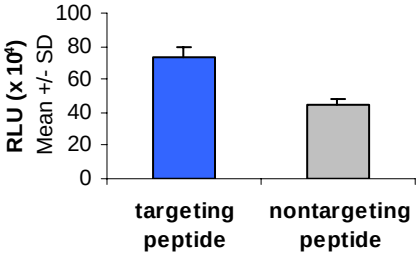

Figure 2: Tranfection efficiency of targeted versus non-targeted nanoparticles (luciferase assay)

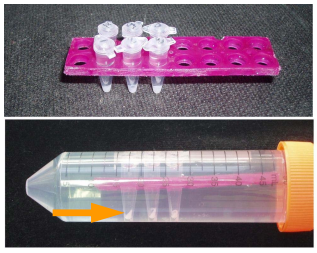

Figure 3: Eppendorf rack
Side views of the rack and falcon tube with cell pellets visible (arrow).

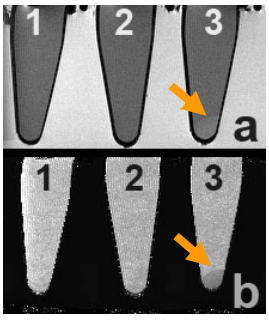

Figure 4: Tumour cell pellet MRI
a) 2D spin-echo of three eppendorfs
1. cells + medium
2. cells + unlabelled nanoparticle
3. cells + Gd-labelled nanoparticle
There is signal enhancement in pellet no 3 (arrow). TR=1.5s, TE=10.9ms

b) T1 map of above pellets, showing decrease in T1 of pellet no 3 (see also Fig 5).

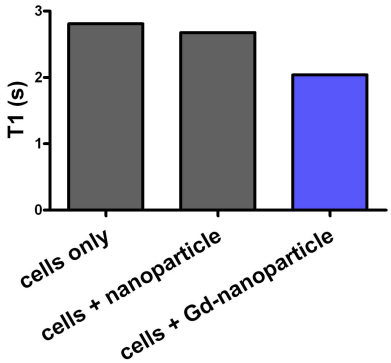

Figure 5: T1 values of tumour cell pellets

Conclusions:

We present a pilot study investigating the MR properties of the complex we have developed. The targeting moiety has increased transfection efficiency, while the Gd3+ moiety exhibits positive contrast in transfected cells. The nature of our nanoparticles allows for extensive optimisation of MR contrast enhancement and gene delivery. In the future we plan to use this complex for non-invasive real-time MR-based monitoring of the specific delivery of gene therapy to tumours in an animal model.

References: 1. Lanza *et al*. J Nucl Cardiol. 2004;11:733-43