# FUNCTIONALIZING POLY(ETHYLENEIMINE) DNA CARRIERS WITH GD-DOTA CONTRAST

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**INTRODUCTION** The poly(ethyleneimine) (PEI) is the one of the most widely used polymers in fabrication of non-viral DNA-carrying vectors. A number of PEI-based carriers have been designed and deployed in many applications despite its lack of biodegradability [1,2]. Yet, to date there was no report on PEI-based gene-carrying vectors conjugated with an MR contrast agent. Recently we developed a method for labeling DNA-encapsulating PEI polyplexes with the MRI contrast agent Gadoterate meglumine (Gd-DOTA), and conducted a proof-of-principle imaging cell transfection study. The effectiveness of the PEI and DNA condensation depends on several parameters, including the nitrogen (polymer) to phosphate (DNA) (N:P) ratios and the number of available primary amines in PEI monomers. The N:P ratio determines the amount of PEI per the amount of DNA, and influences the strength of DNA/PEI condensation, toxicity, and transfection capability of polyplexes. When functionalized with the contrast such as Gd-DOTA, the number of reactive primary amines is reduced. Therefore, the Gd-DOTA:PEI ratio will also play the role in condensation efficiency. In this study, we attempt to demonstrate the feasibility of decorating of DNA-encapsulating PEI polyplexes with MR Gd-DOTA contrast agent without significant reduction in gene-expression with an added capability of observing the cells transfected with Gd-DOTA-decorated DNA/PEI polyplexes using the MR imaging in-vitro.

**MATERIALS AND METHODS** In the **first phase** of the study, Gd-DOTA functionalized DNA-encapsulating PEI polyplexes were synthesized. It was done in three steps. Initially, DOTA-mono-NHS ester (Microcyclics, Dallas, TX) was **conjugated** with high-molecular weight PEI (25 kDa) monomers via simple mixing in three DOTA:PEI amount ratios -- 1:1, 1:10, and 1:100. In each case 12 mg of DOTA-NHS ester (30 µmole of DOTA) was mixed with 3.9 mg (30 µmole), 39 mg (300 µmole), and 390 mg (3 mmole) of PEI dissolved in 1 g/100 ml deionized water solution yielding 390 µl, 3.9 ml, and 39 ml respectively. Following the conjugation, the solutions were run through PD-10 columns with the exclusion limit of 5000 (GE, Healthcare, Piscataway, NJ) in order to remove unconjugated DOTA-mono-NHS ester. Subsequently, that conjugation had indeed taken place was confirmed by taking the NMR of DOTA-PEI samples (data not shown). In the second step, in order to insert Gd into the "DOTA basket," the DOTA-PEI conjugates have been **complexed** with $Gd_2O_3$ (Sigma-Aldrich, St. Louis, MO). This was done by adding $Gd_2O_3$ into the DOTA-PEI solutions drop-wise to avoid drastic pH changes, and stirring the reaction for 24 hours at 60 ºC. Following the complexation with $Gd_2O_3$, solutions were once again run through the PD-10 column to remove any uncomplexed Gd oxide from the solution. The subsequent xylenol orange test for free Gd was negative. Finally the product, Gd-DOTA-PEI complex was **condensed** with green fluorescent protein (GFP)-encoding plasmid DNA forming nanoscale polyplexes at the following N:P ratios, 5, 10, 20, 40, 50, 60, 80, 100, 150, and 200. Following the condensation, samples were run through the DNA-staining gel to confirm absence of free DNA (data not shown). In the **second phase** of the study, NIH 3T3 murine fibroblasts were transfected by synthesized polyplexes, and varying expression of the GFP as well as toxicity levels were observed 8 to 24 hours later depending on Gd:PEI and N:P ratios using fluorescence microscopy and flowcytomery. Finally, in the **third stage** of this work, MR imaging of the solutions with transfected cells was performed to assess the concentration of Gd-DOTA in transfected cells qualitatively as well as quantitatively. To this end, first, the samples with the largest number of GFP-expressing cells for all Gd:PEI ratios were identified using fluorescence microscopy, and the N:P ratio for those samples was noted. Three samples with this N:P ratio and Gd:PEI ratios of 1:1, 1:10, 1:100 were diluted in such a way that predicted concentration of Gd-DOTA in them would be approximately 0.1 mM, 0.4 mM and 0.4 mM respectively. These solutions were places in 200 µl Eppendorf tubes alongside with three Gd-DOTA controls with concentrations 1 mM, 0.5 mM and 0.1 mM and imaged in the Bruker 9.4T animal system using an inversion recovery spin echo sequence with the following imaging parameters: TR=2000 ms, TE=4.8 ms, TI=50:10:600 ms, FOV=$30^2$ mm, Matrix=$128^2$.

**RESULTS** The MR images of the samples with cells transfected by Gd-DOTA DNA-encapsulating PEI polyplexes (left column) and those of controls (right column) is shown in the Figure 1. Comparing the null points for controls and the samples, it can be observed that the concentration of the 1:1 and 1:10 samples are less than 0.5 mM, while that of 1:100 is slightly less than 0.1 mM, as predicted by the deliberate dilution. When plotted as a function of the inversion time (Figure 2), the MR signal shows the null points for samples (solid lines) and controls (dashed lines). Finally, a fluorescence image (Figure 3) shows two typical distributions of GFP-expressing fibroblasts. Of a particular interest here is an observation that cells expressed less of the GFP in $Gd^-$-$DOTA^+$ cells at any N:P ratio than under the $Gd^+$-$DOTA^+$ condition, and about the same amount of the GFP as in controls. These qualitative observations have been validated by flowcytometry (data not shown).

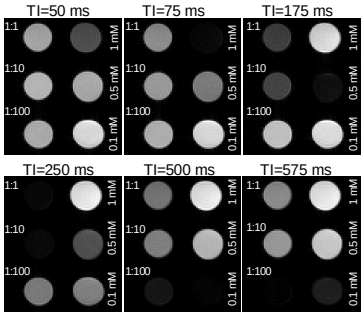


FIGURE 1

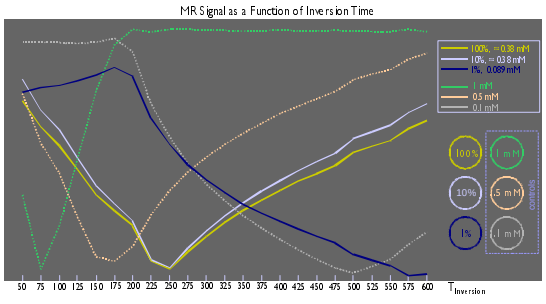


FIGURE 2

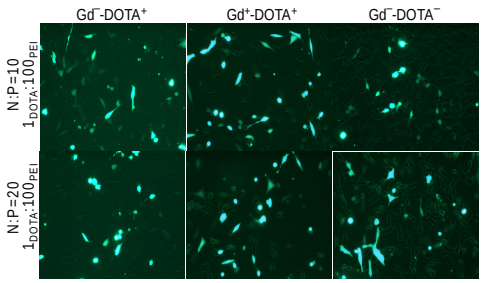


FIGURE 3

**CONCLUSIONS** The experiments in this study have demonstrated a feasibility to functionalize nucleic-acid encapsulating PEI polyplexes with MR Gd-DOTA contrast, and observe them using MR imaging. The cells transfected with Gd-DOTA functionalized PEI polyplexes continued to express GFP at the levels comparable to those without Gd-DOTA. This finding is quite encouraging as it indicates relatively low toxicity, as judged by sustained number of GFP-expressing cells at the levels of the MR contrast concentrations sufficient to generate adequate MR signal change.

**REFERENCES**

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