# Novel MRI Active Gadolinium Liposomes for Cell Labelling

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## Introduction

- Liposomes are artificially constructed spheres of amphipathic lipids that self assemble to form bilayers which enclose an aqueous compartment in the presence of water.
- The properties of liposomes can be altered based on adjusting the physical parameters of the constituent lipids (i.e. charge, head group, tail group). The aqueous interior can be loaded with a variety of molecules (nucleic acids or drugs) to render therapeutic functionality to the liposomes.

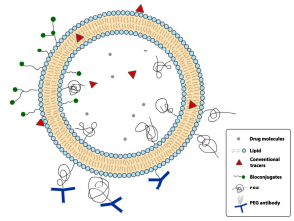
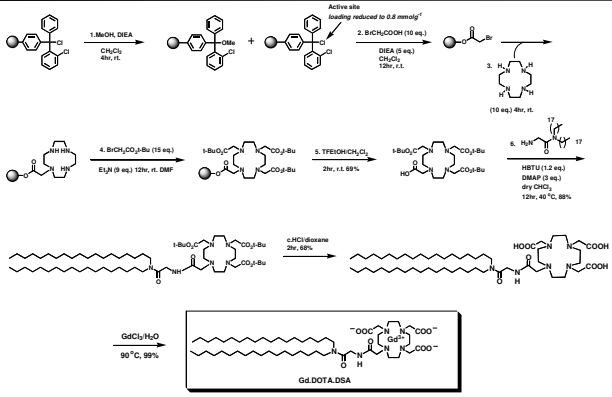


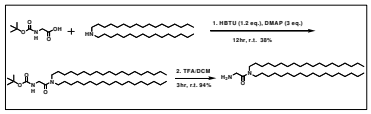

***Figure 1.*** *Liposomes are tuneable delivery vehicles*

- Liposomes offer potential as diagnostic imaging agents given their biodistribution versatility; the incorporation of Gd lipids into a liposome formulation produces paramagnetic liposomes that can be used as targetable contrast agents in MRI.

## Synthesis of Gadolinium Lipid




***Scheme 1.*** *Synthesis of Gd.DOTA.DSA*




***Scheme 2.*** *Synthesis of precursor amine*

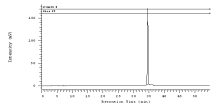


***Figure 2.*** *HPLC trace of pure Gd.DOTA.DSA*

## Aims and Objectives

- The aim of this project has been to design and synthesise novel Gd lipids and to formulate Gd liposomes for *in vitro* cellular MRI labelling, and *in vivo* tracking of transplanted cells.
- The uptake efficiency of these liposomes can be assessed by finding an optimised cationic liposome formulation and hence the correct balance between the ratio of cationic, neutral and Gd lipids. This is done by the incorporation of a fluorescence probe in the liposome formulation and subsequent fluorescence uptake assays. The cell viability and cytotoxicity is also investigated along with the transfection ability of the optimised formulation with plasmid DNA.
- Gd liposome pre-labelling and MRI visibility of functional cells such as neutrophils (lung inflammation models) and stem cells (brain stroke legions/cardiac infarcts) will be investigated. The transfection ability of Gd liposomes for gene correction will also be studied in relevant models.

## In Vitro Investigations

- A series of cationic liposomes were formulated using various ratios of CDAN, DOPE, Gd.DOTA DSA lipids and a small amount of a fluorescent lipid (Table 1). Liposomes were incubated with HeLa cells and the amount of liposome and thus Gd lipid uptake into cells was determined from the fluorescence emission intensity of the cell lysate.
- In addition, cell viability (MTT assay) and cytotoxicity (LDH assay) was assessed in order to determine which liposome had the best uptake/toxicity profile.
- Using this criteria liposome 2. was selected for imaging studies.

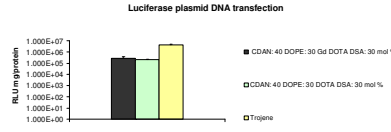
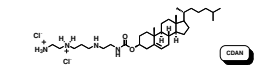


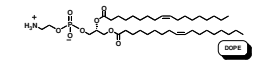

***Figure 3.*** *Plasmid DNA transfection with an optimal formulation.*

| Liposome no: | CDAN mol % | DOPE mol % | Gd.DOTA.DSA mol % |
|---|---|---|---|
| 1 | 30 | 30 | 40 |
| control 1 | 30 | 30 | DOTA.DSA 40 |
| 2 | 40 | 30 | 30 |
| control 2 | 40 | 30 | DOTA.DSA 30 |
| 3 | 50 | 0 | 50 |
| control 3 | 50 | 0 | DOTA.DSA 50 |
| 4 | 20 | 20 | 60 |
| control 4 | 20 | 20 | DOTA.DSA 60 |
| 5 | 40 | 30 | Gd.DOTA.Chol 30 |
| control 5 | 40 | 30 | Cholesterol 30 |

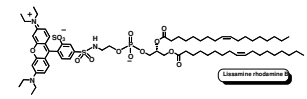
***Table 1.*** *Liposome formulations tested.*




**CDAN: cationic lipid; facilitates endocytosis**




**DOPE: neutral helper lipid; aids in endosomal disruption**




**DOPE-Rhodamine: Fluorescence uptake studies and microscopy**

***Figure 4.*** *Lipids used in liposome formulations along with Gd Lipid.*

## MRI Analysis

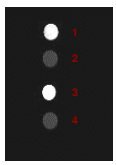



***Figure 5.*** *Phantom MR images of Gd.DOTA.DSA with controls. Coronal $T_1$ weighted images with the following parameters: TR = 700, TE = 15 ms, FOV = 70 x 70 cm², averages = 10, and matrix = 256 x 256 (sems sequence used to obtain $T_1$ values with 11 TR's) 4.7T.*

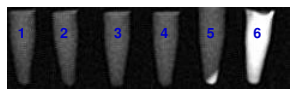



***Figure 6.*** *MR image of cell experiment.* Sagittal $T_1$ weighted images with the following parameters: TR = 700, TE = 15 ms, FOV = 60 x 60 cm², averages = 4, and matrix = 256 x 128 (sems sequence used to obtain $T_1$ values with 11 TR's) 4.7T.

| Sample no. | Content | $T_1$ (ms) |
|---|---|---|
| 1 | 0.5 mM Gd.DOTA.DSA in $H_2O$ | 400.1 ± 15.92 |
| 2 | 0.5 mM DOTA DSA in $H_2O$ | 2356 ± 112.588 |
| 3 | Magnevist ® in $H_2O$ (equivalent Gd atom content as sample 1) | 371.0 ± 4.321 |
| 4 | $H_2O$ | 2750 ± 121.8 |

***Table 2.*** *Phantom MRI studies of new Gd lipid.*

| Sample no. | Content | $T_1$ (ms) |
|---|---|---|
| 6 | Gd Liposome | 311.4 ± 40.10 |
| 5 | Gd Liposome + HeLa cells | 688.6 ± 53.60 |
| 4 | Control Liposome | 2248 ± 212.0 |
| 3 | Control Liposome + HeLa cells | 2018 ± 132.8 |
| 2 | HeLa cells | 1998 ± 163.7 |
| 1 | PBS | 1958 ± 146.2 |

***Table 3.*** *MR images of cell pellet and controls.*

- The $T_1$ value of the Gd.DOTA DSA phantom compares excellently with that of the commercial contrast agent Magnevist®, the uptake and relaxation efficiency of the Gd liposome is verified by the bright cell pellet image in tube 5 (figure 6).

## Fluorescence Microscopy

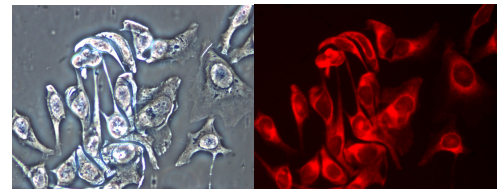


***Figure 7.*** *Fluorescence Microscopy Results (40 x magnification) Left Image: phase contrast. Right image: Fluorescence*

- The fluorescence microscopy results show that Gd liposomes are taken up into HeLa cells and the intensity of the fluorescence is quite high.
- The image on the right of liposome formulation no.2 shows that the liposomes are ubiquitously present inside the cytoplasm of the cell, and are aggregated around the outer nuclear membrane. This also confirms that the mechanism of cell entry is potentially via endocytosis.

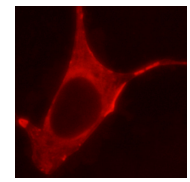


***Figure 8.*** *Liposome uptake is perinuclear.*

## Conclusion

- Efficient uptake of Gd liposomes into HeLa cells was shown. Phantom MRI analysis of Gd.DOTA DSA revealed this lipid to be as effective as the commercial contrast agent Magnevist®.
- MRI analysis of HeLa cell pellets labelled with Gd liposome produced a brightly visible mass of cells; reducing their $T_1$ value relative to control cells by 66%, and toxicity was minimal.
- Intracellular uptake of liposomes was verified by fluorescence and confocal imaging.
- DNA transfection with liposomes formulated with Gd.DOTA DSA gave comparable transfection results to the commercial transfection agent Trojene® (Avanti Polar Lipids inc. USA).

## Future Directions

- The cellular uptake of the Gd liposomes using primary cell lines and their cellular biodistribution *in vivo* following transplantation or transfusion will be investigated i.e. cell migration and trafficking.