Paramagnetic Labeling of Cells Using High Relaxivity Nanoparticles

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Introduction

Magnetic labeling of cells allows for in vivo monitoring of cellular migration and trafficking following transplantation or infusion using MRI. Previously used cell labeling contrast agents included dendrimer-based gadolinium chelates (1), magnetodendrimers with iron coatings (2,3), superparamagnetic iron oxides with transfection agents (4), and dextran linked gadolinium derivatives (5). We have investigated the first use of the paramagnetic properties of gadolinium incorporated into polymerized lipid-based nanoparticles (Gd-NP). These non-toxic T1 contrast agents can be used for monitoring cell trafficking in vivo.

Methods

Synthesis of Nanoparticles: The Gd-NPs were prepared using purified lipid components (45%PC, 30%Gd-lipid, 25% cationic lipid) (6). The lipids were dissolved in CHCl3 and CH3OH and the solvents were evaporated. The residue was dried in vacuo for 24h while shielded from light. Distilled and deionized water was added to yield a heterogeneous solution 30 mM in lipid concentration. The lipid/water mixture was then sonicated with a probe-tip sonicator for approximately one hour and the pH of the solution was maintained between 7.0 and 7.5. The nanoparticles were polymerized by transferring the solution to a petri dish cooled to 0 ˚C, and irradiated at 254 nm for approximately one hour. The NPs were then filtered through a 0.2 µm filter and collected.

Cell Labeling and Imaging: Three different concentrations of Gd-NPs were used for cell labeling: condition A used a Gd(+3) concentration of 111 uM; condition B used a Gd(+3) concentration of 219 uM; condition C used Gd(+3) concentration of 429 uM. These solutions were added to 60% confluent HeLa cells in 100mm diameter culture dishes containing 10 mL RPMI1640 culture medium supplemented with 10% fetal calf serum. The labeling was carried out overnight in the cell culture incubator. A total of 9 dishes of control Hela cells were cultured to obtain the unlabeled cells. Before harvest, cells were washed three times in PBS, counted, placed in test tubes, and pelleted. Each test tube contained 5 x 10^6 cells topped with 4% gelatin. Labeled cells were titrated into unlabeled cells in four test tubes and were imaged for each of the three incubation conditions (A-C). The ratio of labeled to unlabeled cells were 0% (control), 1%, 10% and 100% while maintaining a total of 5x10^6 cells in each tube. MRI was performed at 1.5 T using a T1-weighted SE sequence (TE min, TR 300 ms, 256x128, slice thickness 3 mm). The concentration of Gd(+3) in each test tube containing the imaged cells was determined by ICP-AAS following acid digestion (Desert Analytics, Tuson, AZ).

Results

Relaxivity Measurements (R1): The R1 value of the Gd-NPs is 5.7 $s^{-1}mM^{-1}$ at 4.2 MHz, 6.2 $s^{-1}mM^{-1}$ at 42 MHz and 5.5 $s^{-1}mM^{-1}$ at 63 MHz . The expected decrease in the relaxivity at high frequency (63 MHz) as opposed to low frequency (4.2 MHz) was not observed. This implies a characteristic peak in the NMRD profile as would be expected for macromolecular paramagnetic agents of this size (40-60 nanometers).

MR Image and Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS) Analysis of Gd-NP Labeled Cells:

Figure 1 shows the T1-weighted MRI of the labeled HeLa cells. There is a 70 % increase in signal intensity (SI) in the 100% labeled cells vs unlabeled controls (column B rows 1 and 4). This increase was also observed when cells were incubated with condition C. It implies that the uptake of the Gd-NPs had reached saturation at concentrations of Gd(+3) above 219 uM. This was confirmed by gadolinium analysis of the cells using ICP-AAS. The total amount of Gd(+3) in 100% labeled cells was found to be approximately 45 nmoles (B and C). In contrast, SI increase is 48% from control to 100% labeled cells under condition A ([Gd(+3)]=111 µM). It corresponds to decreased Gd-NP concentration used in cell incubation. The amount of Gd(+3) ions in the cells was found to be approximately 38 nmoles under condition A, confirming the observed decrease in signal intensity. The titration of labeled cells with unlabeled cells results in an expected decrease in the SI as shown in Figure 2a-c. Using labeling conditions B and C, the SI is visibly and statistically ($P<0.5$) different than controls even when titrating the cells a 100 fold (compare column B and C, rows 2 and 3 to controls in row 4 in Fig.1). These results indicate that even at high cell dilutions (1% labeled), the Gd-NP labeled cells are easily detected by MRI at clinically relevant field strengths. The labeling efficiency for condition A, B, and C were 3.42%, 2.05%, and 1.05% respectively.

Conclusion

The ability to detect by MRI intra-cellular Gd-NPs and cell distribution is an important consideration for in-vivo tracking of dividing cells and for stem cell research. The capability of using T1 agents to label cells efficiently and be detected at relatively low concentrations opens the door for high anatomic resolution to be coupled with clear regions of change in T1 contrast to detect the presence of these cells. In addition, these systems can be added to optical probes as paramagnetic labeling does not interfere with these processes. These techniques and materials will be useful for answering critical questions in cell trafficking in biology.

References

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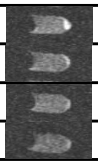
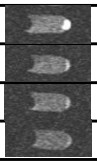
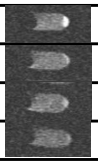
	A	B	C	
1				100% labeled cells
2				10% labeled cells
3				1% labeled cells
4				0% (control)
Gd(+3)	111uM	219uM	429uM	

Figure 1. T1-weighted images of cell pellets labeled with Gd-NPs. Column A through C represents labeling conditions A-C (see text for details). Rows 1-4 are titrations of paramagnetic labeled cells with unlabeled cells while maintaining total cell concentration of 5x10^6.

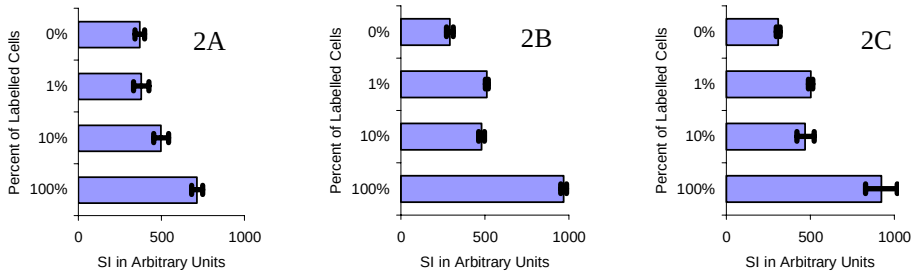


Figure 2. Signal intensity (SI) plot of cell pellets shown in Figure 1. Y-axis represents steps of 10 fold serial titration of the labeled cells, while maintaining the total cell count constant. 2A, 2B, and 2C corresponds to labeling conditions A, B, C shown in Figure 1.