Dual-Labeled Nanoparticles for Studying Cell Migration and Trafficking via Optical and Magnetic Resonance Imaging

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Introduction

Monitoring cellular migration and trafficking in vivo is important for studying stem cell distribution, cancer metastasis, and various physiologic and pathologic processes. Many techniques for the study of cell transplantation require histological analysis to determine the fate and migration of cells. However, these techniques lack the ability to serially track the full body distribution of the transplanted cells (1-3). More recently, magnetic labeling of cells allow the in vivo tracking of implanted cells via MRI, however, these techniques lack the ability to characterize the transplanted cells at a cellular level (4 -5). We report the first use of a dual-labeled gadolinium (Gd^{3+})-rhodamine (Rd) polymerized nanoparticle (Gd-Rd-NP) that achieves both these purposes. These fluorescent MR contrast agents can potentially be used for serial monitoring of cell trafficking in vivo, MRI-guided tissue procurement, and detailed cellular characterization via optical imaging.

Methods

Synthesis of Nanoparticles: Purified polymerizable lipid (PL) components (50%PC-PL, 29%Gd-PL, 1% Rd-Lissamine, 20% DOTA) were used for the construction of the Gd-Rd-NPs (6). The lipids were dissolved $CHCl_3$ and CH_3OH and the solvents were evaporated. The residue was dried in vacuo for 24h while shielded from light, and was diluted to a 15 mM lipid concentration in deionized water. The lipid/water mixture was then sonicated with a probe-tip sonicator for approximately two hours and the pH of the solution was maintained between 7.0 and 7.5. The Gd-Rd-NPs were polymerized by transferring the solution to a petri dish cooled to 0 ˚C, and irradiated with UV light (254 nm) for approximately two hours. The NPs were then filtered through a 0.2 µm filter and collected.

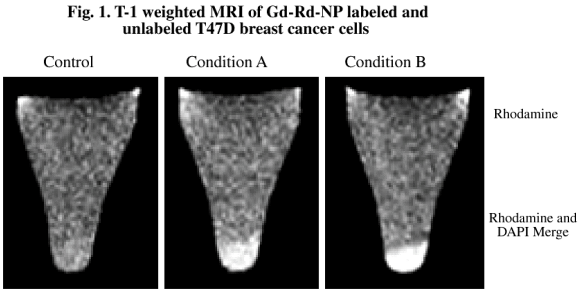
Cell Labeling and Imaging: Cell labeling was performed by incubating the Gd-Rd-NPs with 60% confluent breast cancer cells T47D in 100mm diameter culture dishes containing 5mL of RPMI1640 culture medium supplemented with 10% fetal calf serum. Two different conditions of Gd-Rd-NPs were used for cell labeling: Condition A used a concentration of nanoparticles yielding 348 uM Gd^{3+} and 12 uM Rd; Condition B used a nanoparticle concentration yielding of 1.4mM Gd^{3+} and 48uM Rd. The labeling was carried out for 4 hours in a cell culture incubator. Control T47D samples were not incubated Gd-Rd-NPs and were cultured under the same conditions. Before harvesting, cells were washed three times in PBS, then trypsinized and washed again in the 15ml tubes with PBS. Cells were then counted, placed in 1.5 mL Eppendorf tubes, and pelleted. Each test tube contained ~1.5 x 10^6 cells topped with 500ul of 4% gelatin. These pellets were imaged via MRI at 7 T using a T1-weighted SE sequence (TE min, TR 300 ms, 256x128, slice thickness 3 mm). In parallel, the uptake of the NPs was also visualized using fluorescent microscopy. The T47D cells were plated in cultured chambers and then incubated for four hours at the same conditions (Conditions A&B) described above. After the incubation, the cells were washed and fixed in neutral 4% paraformaldehyde-PBS for 20min at room temperature. Before fluorescent microscopy visualization, the nuclei were counterstained by DAPI for cell viability.

Results

Size, Zeta Potential: The Gd-Rd-NPs were ~73 nm in diameter with a polydispersity of 0.249 (determined by dynamic light scattering). The zeta potential was also determined to be ~62.97 mV (ZetaPALS, Brookhaven Instruments, NY).

Magnetic Resonance Imaging of Gd-Rd-NP Unlabeled and Labeled T47D Cells: Figure 1 shows the T1-weighted MRI of the labeled and unlabeled T47D cells. The images show a concentration dependent uptake of Gd-Rd-NPs. The pellet of cells incubated with condition A show a 139% increase in average relative pixel intensity versus control, while the pellet of cells incubated with condition B show a 146% increase in average relative pixel intensity versus control.

Fluorescent Microscopy Imaging of Gd-Rd-NPs Labeled and Unlabeled T47D cells: Under fluorescent microscopy, it was observed that the Gd-Rd-NPs accumulated in the cells after only four hours of incubation. Similar to the MR images, Figure 2 demonstrates that intensity of Rhodamine fluorescence and NP uptake into the cells is directly proportional to the labeling concentrations of the Gd-Rd-NPs (Condition A vs B). We have also observed that NPs are endocytosed quickly by the T47D cells and that these NPs evenly distribute in the cytoplasm. Positive DAPI staining was demonstrated in both labeled and unlabeled cells indicating cell viability after Gd-Rd-NP labeling.



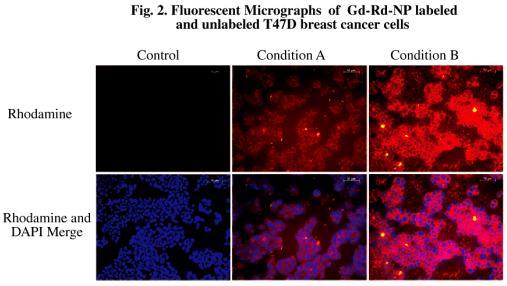


Figure 1. T1 Weighted MRI Images of ~1.5 x 10^6 T47D cells were labeled with Condition A and B or unlabeled(control). The average relative pixel intensity of the labeled pellets versus control increases with increasing labeling concentrations of Gd-Rd-NPs.

Figure 2. Uptake of Gd-Rd-NPs in T47D cells in vitro. After four hours of NP incubation,T47D cells demonstrate significant amount of Rhoadmine flourescene (RED). The amount of NP uptake into cells is directly proportional to the labeling concentration of the NP (see Methods). DAPI positive counterstaining (BLUE) indicates the viability of both NP labeled and unlabeled (control) cells.

Conclusion

We have constructed a new dual fluorescent labeled MRI nanoparticle from simple monomers. These dual-labeled Gd-Rd-NPs can be used to efficiently and quickly label cells that could be detected by MRI and optical imaging. These dual labeled NPs offer the advantage of combined in vivo imaging of cells as well as detailed histological analysis. Cells labeled with Gd-Rd-NPs are viable and can be localized by MRI. We now plan to investigate the in vivo localization of labeled transplanted cells, MRI-guided tissue biopsies of cell migration sites, and optical enabled characterization of cellular morphology.

References

1. Jackson KA. J Clin Invest. 2001(107)1395-1402; 2.Orlic D. Nature 2001(410)701-5; 3. Toma C. Circulation 2002 (105) 93-8; 4. Frank, JA Acad Radiol 2002; (9); S484-S487; 5. Kraitchman DL. Circulation 2003; 107:2290-3; 6. Storrs RW. J Am Chem Soc 1995 (117)