Multi-colored Nano-CEST MRI Contrast Particles for Cell Imaging

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INTRODUCTION

The labeling of cells with iron oxide particles has been used widely for MRI tracking of transplanted cells in vivo. However it is restricted to imaging one labeled cell population at each experiment. Chemical Exchange Saturation Transfer (CEST) agents sensitive to different saturation frequencies (so-called multi-color agents) can be designed, and we were interested in testing whether such agents can be used for imaging simultaneously a number of transplanted cell populations. One issue is how to load the cells with such agents. Paramagnetic ions have previously been incorporated into liposomes [1-3]. More recently nanoparticles have been used for fluoro[4] and PARACEST[5] contrast agents. By incorporating CEST agents into liposomes, we can limit their interactions with biological media and avoid local dilution of agent. We demonstrate that multi-color CEST nanoparticles can be taken up by 9L glioma cells during incubation at measurable concentrations, with no toxic effect (as seen by a cell viability assay).

METHODS

Preparation of Fluorescent CEST Liposomes Liposomes composed of phosphatidylcholine (PtdCho) and cholesterol (1:1 mole ratio) and nitrobenzoxodiazol (NBD) labeled lipids (1.8 total mole %) were formed with the extended hydration method[6] using a starting solution containing the appropriate CEST Poly Amino Acid . Poly-L-Lysine (13.8kD, 25kD, 45kD and 290kD), Poly-L-Ornithine (9kD) and Poly-L-Arg (35.5kD) were purchased from Sigma and diluted to 25 mg/mL in PBS (0.01M, pH 7.4). Subsequent solutions used to prepare the liposomes came from this stock. After liposomes were extruded, unencapsulated Poly Amino Acid was removed by dialysis using 250kD cutoff PVDF dialysis tubing (Spectrum Laboratories, Inc). Size and concentration of liposomes were then measured using dynamic light scattering (Nanosizer, 90ZS, Malvern Instruments) and fluorescence (Victor V, Perkin Elmer). The final stock of liposomes have a particle concentration of ~100 nM and average liposome sizes were ~140 nm for PLL liposomes, 250 nm for PLArg and 150nm for PLOrn all at pH 7.4.

NMR Experiments All experiments were performed at 310K on an 11.7T Bruker Avance system using a triple axis gradient NMR probe. Z spectra (relative water saturation as a function of RF irradiation frequency) were taken with a saturation transfer sequence consisting of a saturation pulse with variable offset, power and time followed by spin-echo acquisition. Hard 10μs (90˚) and 20μs (180˚) pulses were centered on the water resonance. QUEST [7] experimental data were collected using a 5μT saturation pulse, with saturation frequencies of +/- 3.65 ppm from water, varying the saturation time between 1 – 10 s.

Cell Culture Experiments For in vitro experiments, 9L rat glioma cells were seeded in 96 well plates with ~1.38e5 cells per well. These were allowed to attach overnight, and serial dilutions of liposomes were added to the plates. MTT cell viability assays (Sigma) were performed according to the manufacturer's protocol.

RESULTS AND DISCUSSION

Initial experiments were carried out in order to optimize the contrast as a function of both concentration and size of CEST proteins. A series of dilutions of Poly-L-Lysine (25 kD) were used to produce liposomes, with 18mg/mL determined to be optimal. Next a series of liposomes were produced with Poly-L-L-Lysine sizes varying from 13kD to 290kD at a concentration of 18 mg/mL. Finally, a series of different proteins were studied at this concentration, Poly-L-Arginine, Poly-L-Ornithine, and Poly-L-Lysine. In figure 1 on the left the contrast generated by two different protein encapsulated liposomes is plotted as a function of irradiation frequency, displaying the two different colors for Poly-L-Ornithine(particle concentration= 110nM) and Poly-L-Arginine (35nM).

The next step was to determine a method that could be used to insert these liposomes into cells, and test whether or not the resulting cells remained viable. 9L rat glioma cells were incubated overnight in 96 well plates with a series of liposome concentrations in the growth media. In figure 2, we show a merged image displaying the incorporation of fluorescently labeled liposomes into the 9L cells. In addition, a plate reader was used to quantitatively determine how much of the 13kD PLL liposomes were incorporated into cells via this fluorescence. In figure 3, the results of an MTT assay are shown as a function of liposomal concentration for overnight ~ 20 hr incubation of liposomes. For this level of confluent cells (~25%), concentrations of 0.317 nM Poly-L-Lysine liposome (18mg/mL Poly-L-Lysine) in the media were tolerated, producing viable cells.

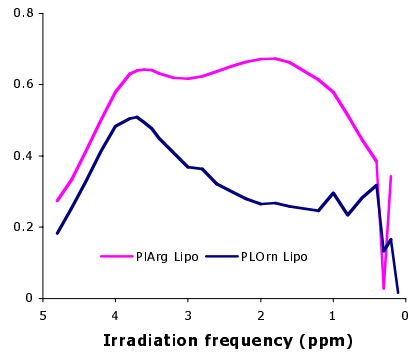

Figure 1. Experimental Frequency Dependence of Contrast for Liposomes

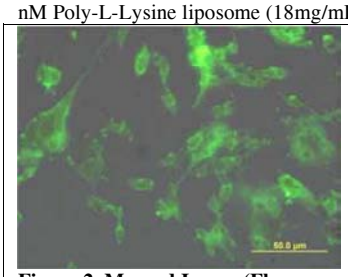

Figure 2. Merged Image (Fluorescence/Bright Field) of 9L cells with nbd labeled Liposomes

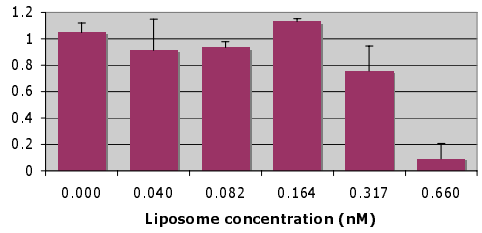

Figure 3. MTT Cell Viability Assay for 9L Cells vs. Liposome concentration

CONCLUSIONS

In this study we have shown that nanometer sized CEST liposomes could be produced from protein solutions, and that these displayed significant contrast at nM concentrations of liposome. The multicolor characteristics of this contrast is preserved upon encapsulation. In addition, these agents can be incorporated into cells in culture via incubation, with the resulting cells viable according to a standard viability assay. This new agent should prove useful for future cell tracking studies.

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