Complexation of MPIO with poly-l-lysine greatly enhances magnetic cell labeling efficiency

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INTRODUCTION:

MRI offers the potential to noninvasively track cells in vivo using innovative approaches to cell labeling and image acquisition. Cells loaded with iron oxide particles generate a pronounced contrast in T2- and T2*-weighted images. Strategies have been developed for labeling cells with micron sized iron oxide particles (MPIOs) for in vivo visualization of cells by MRI (1). Although this approach is well established and has a variety of applications, current protocols employ long labeling times. This is problematic for cells which do not fare well for long periods of time in culture, such as neutrophils and hepatocytes.

It has been previously demonstrated that incubation of negatively charged iron oxide nanoparticles with positively charged transfection agents, such as poly-l-lysine (PLL) increases labeling efficiency (2). Therefore, it was hypothesized that pre-incubating MPIOs with various quantities of PLL would similarly enhance the rate of magnetic cell labeling.

MATERIALS AND METHODS:

The short-term labeling efficiency and toxicity of different MPIO/PLL formulations and COOH and NH_2 modified MPIOs, were compared in mouse embryonic fibroblasts. MPIOs used are 1.63 µm diameter, polystyrene/divinyl benzene coated (Bangs Laboratories). MPIO #2 is a silica coated 1 µm particle from Chemicell. Different formulations of MPIO/PLL were made through simple incubation of PLL and MPIOs in dH_2O for 3 hours. Commercial MPIOs and MPIO/PLL formulations were characterized by measuring zeta potential using Phase Analysis Light Scattering. The labeling efficiency of each particle was assessed by simple incubation for 1-4 hours and counting labeled cells via flow cytometry. The toxicity of each particle formulation was simultaneously assessed using Sytox Blue Dead Cell Stain (Invitrogen). Fluorescence microscopy was used to confirm data from flow cytometry.

RESULTS:

Zeta potential of COOH modified MPIOs, as well as silica coated MPIOs was highly negative. Interestingly, the NH_2 coated MPIO also had negative zeta potential. All formulations of MPIO/PLL had positive zeta potential, except for the most dilute sample. As expected, increased amounts of PLL resulted in more positive charge. Flow cytometry data of STO labeling shows higher labeling percentages with all formulations of MPIO/PLL particles than with MPIO COOH, for all time points (Figure 1). At 2 hours, labeling percentages for all MPIO/PLL formulations plateau at ~80% of cells, with very little increase in labeling percentages at 3 and 4 hours. MPIO COOH and MPIO #2 particles have the worst labeling performance, labeling up to ~20% of cells by 4 hours. MPIO NH_2 particles have higher labeling percentages than MPIO COOH, but lower than all the MPIO/PLL particles for all time points. With the exception of MPIO NH_2 and 1:4000 PLL, particles with positive zeta potentials have higher labeling efficiencies. Microscope images clearly verify the results obtained by flow cytometry (Figure 2). Toxicity assessment of each particle formulation shows at least 96% viable cells for all particle formulations.

DISCUSSION:

The results demonstrate that complexation of PLL with MPIOs prior to magnetic cell labeling produces better labeling efficiency than negatively charged MPIOs. All MPIO/PLL formulations label up to 80% of cells in fewer than 2 hours. This was confirmed in images taken with a microscope. The lower labeling percentages at 1 hour for MPIO/PLL formulations were due to particles only associating with cells at the cell surface, which was clear in the microscope images too. This suggests that minimum labeling time for MPIO/PLL is 2 hours. The labeling efficiency of NH_2 MPIOs is also higher than that of the COOH MPIOs, suggesting that the NH_2 groups may have a positive influence on cellular uptake despite the net negative charge on the particle. While 1:4000 PLL MPIO has a small net negative charge, it is clear that the positive charged transfection agent PLL still enhances labeling efficiency just as well in low concentrations. This may be due to the fact that zeta potentials are commonly measured in water, and labeling occurs in cell culture media.

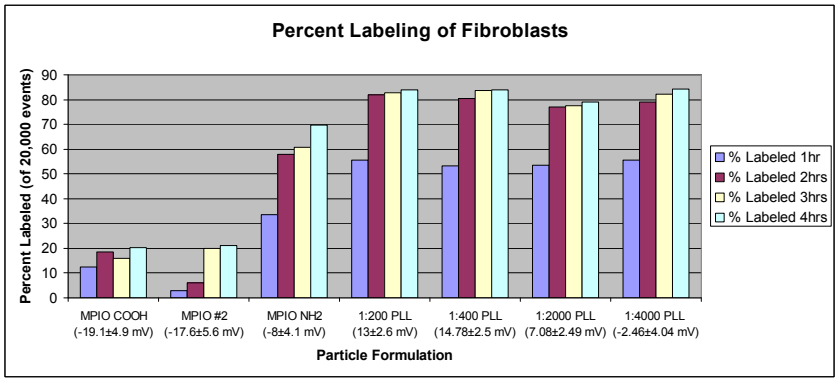

Figure 1: Flow cytometry data of labeling efficiency versus particle formulation. Zeta potential is shown in mV. With the exception of MPIO NH_2 and 1:4000 PLL, particles with positive zeta potentials have higher labeling efficiencies.

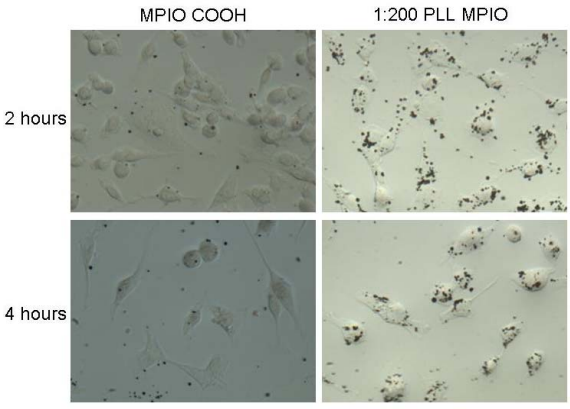

Figure 2: A side by side comparison between STOs mixed with MPIO COOH and 1:200 PLL MPIOs at 4 and 2 hour incubation times. Clearly, there are more 1:200 PLL MPIOs internalized within cells than MPIO COOH particles at even 2 hours incubation time.

References: 1) Shapiro, EM, et al, *MRM* 53, 329-338 (2005). 2) Frank JA, et al, Radiology 228: 480–487 (2003).