Efficient Labeling of Multiple Cell Lines with a New SPIO Agent for Cell Tracking by MRI

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Background: Cellular imaging with MRI has applications in a wide range of areas, including cancer metastasis, stem cell tracking, inflammation, and diabetes. The detection of small numbers of cells, and even single cells, with MRI has been demonstrated and relies on high resolution, high quality imaging in conjunction with intracellular contrast agents. Superparamagnetic iron oxide nanoparticles (SPIO) have been used for many years to label cells for this purpose. SPIO agents are available in a wide range of sizes and surface coatings that affect their biological properties and uptake characteristics. SPIOs are taken up easily by phagocytic cells, but are more difficult to load into non-phagocytic cells. A number of studies have demonstrated the utility of using non-viral transfection agents to enhance SPIO uptake by non-phagocytic cells. Recently the most commonly used SPIO, Feridex, went off market in North America. The purpose of this study was to test a new commercially available SPIO which has a colloidal size of 50 nm, a zeta potential of +31 mV and which is cross-linked with a rhodamine B label. Here we show that a variety of cell lines can be labeled with this agent (MoldayION Rhodamine B, BioPal Inc), by simple co-incubation, without the use of transfection agents, at a level that permits their detection by MRI and without affecting cell viability.

Methods: Three different cell lines were tested: (i) human natural killer cells (KHYG-1), human breast cancer cells (MDA-231BR-Her2) and mouse mesenchymal stem cells (MSC). The 231BR-Her2 were previously transfected with the enhanced green fluorescence protein (EGFP). The MSC were harvested from a transgenic mouse ubiquitous for EGFP. The KHYG-1 cells were labeled with PKH67 a green fluorescent cell linker dye. All cells were labeled at passage 3-9. Labeling was performed by incubation of cells with MoldayION Rhodamine B for 24 hours in culture medium for both adherent (231BR-Her2, MSC) and nonadherent (KHYG-1) cell lines. The cells were incubated in a 6 well plate with an iron concentration of 50μg/mL (adherent cell line) in a total volume of 2mL or 100μg/mL (suspension cell line) SPIO per well in a total volume of 4mL. After incubation unincorporated iron particles were removed by repeated washing with HBSS. Samples of the cells were set aside for (i) preparation of cytospins for staining of iron with Perl's Prussian blue, (ii) fluorescence microscopy, (iii) testing cell viability by the trypan blue exclusion assay and the MTT assay, and (iv) electron microscopy. Cell size was measured before and after labeling. A cell sample was prepared for MRI by placing labeled cells between two layers of gelatin in a single transparent well from a 64 well plate. Imaging was performed with the steady state free precession (SSFP) sequence at 3 Tesla using a custom built gradient coil and solenoidal RF coil the size of the well sample.

Results: Figure 1 shows the PPB (iron is blue) and fluorescence images (rhodamine B of Molday is red, EGFP and PKH are green, DAPI is blue) of all three cells labeled with Molday-Rho. All cells label well with Molday-Rho and the amount of extracellular iron is almost negligible, which is not the case for all other agents we have used in the past in our labs. Labeling efficiency is almost 100% for adherent cells (cancer and stem cells) and above 80% for non-adherent cells (KHYG-1). Viability measured by trypan blue was 82 and 77.5% for labeled and unlabeled MSC, 94.7 and 94.5% for labeled and unlabeled 231BR-Her2 and 89 and 90.3% for labeled and unlabeled KHYG-1 cells (Figure 2). The mean cell size increased with labeling, only slightly for the 231BR-Her1 and KHYG-1 cells, but more substantially for the MSC which were also the most heavily labeled (from 18.2 to 23.7 microns). Electron microscopy shows that the Molday particles are contained within vacuoles or lysosomes in the cytoplasm, an example is shown in Figure 3 for MSC (dark round circles). MRI shows that individual Molday labeled cells can be detected in cell samples, the example shown in Figure 4 is for KHYG-1 which contained the least amount of iron. The MTT assay indicated that labeling with Molday had a small effect on cell viability, the largest effect was on the cancer cells, but that the viable cell population proliferates normally.

Figure 1. PPB (left) and fluorescence (right)

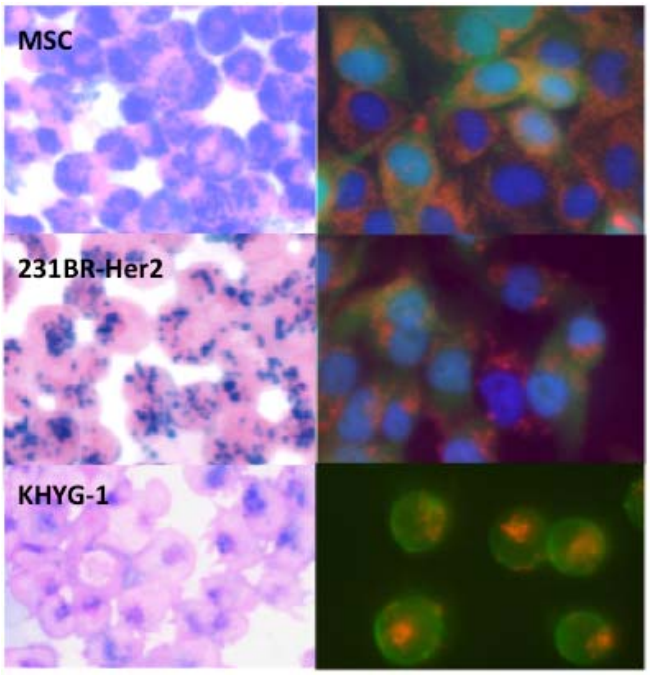

Figure 2.

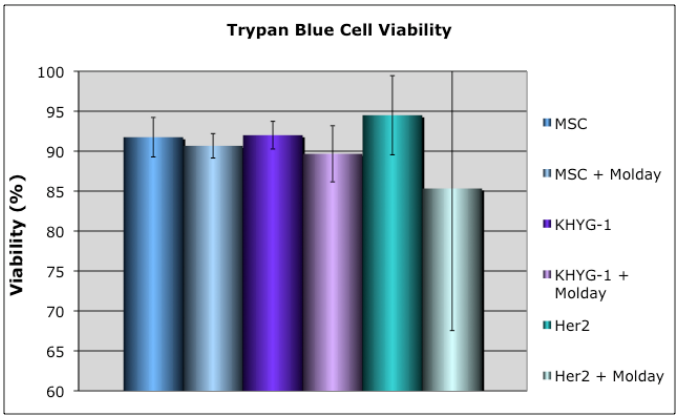

Figure 3. EM of labeled MSC

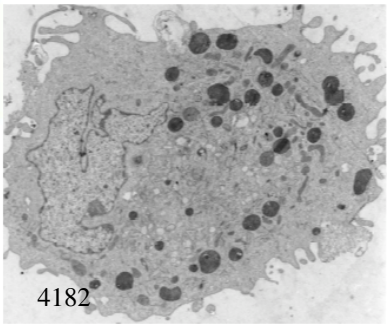

Fig 4. MRI of labeled KHYG-1 cells