Intracellular labeling of mammalian cells by combining FDA approved superparamagnetic iron oxide MR contrast agents and commonly used transfection agents.

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Transfection Agents (TA) mixed with FDA approved superparamagnetic iron oxide nanoparticle (SPIO), effectively coating the SPIO, resulted in endosomal incorporation of iron. (Stem) Cells were incubated with TA-SPIO mixtures and evaluated. Intracytoplasmic particles stained with Prussian Blue were detected and MR images and relaxometry of labeled cells revealed decrease in signal intensity on T2w imaging and an increase of 1/T2. This method will facilitate the use MRI to monitor labeled cells following transplantation in a clinical setting.

Introduction: Magnetic labeling of stem cells (SC) provides the ability to in vivo monitor the migration and trafficking of these cells following transplantation or infusion (1,2). Dextran (Dex)-coated (ultrasmall) superparamagnetic iron oxide ((u)SPIO) nanoparticles, cannot be used to efficiently label stem cells in vitro in their native unmodified form (1,2). Transfection agents (TA) are macromolecules with high net electrostatic charges used for non-viral transfection of DNA into the cell (3). Magnetodendrimer (MD) is a new class of SPIO coated with dendrimer, a known TA, and MD is efficient at labeling cells (4,5). We hypothesized that TAs would cover Dex coated (U)SPIO and chaperon the nanoparticles into cells. This study presents results on magnetic cell labeling using TAs with Feridex or MION-46L that can be used for monitoring cell migration in vivo.

Methods: TA's (Superfect (SF), a heat-activated dendrimer,Qiagen, Valencia, CA), PLUS/ Lipofectamine (Life Technologies, Gaithersburg, MD), and poly-L-lysine (Sigma, St. Louis, MO) were incubated with Feridex (Berlex Laboratories, Wayne, NJ) and MION-46L for 60 min in cell culture medium at various different concentrations. Human mesenchymal stem cells (MSC), mouse T-cells (ML), rat oligodendrocyte progenitor (CG-4) cells, and human cervical carcinoma (HeLa) were incubated from 4 to 48 hours with 25 μg Fe/ml TA-coated iron oxides. Cellular labeling was evaluated using Prussian Blue (PB) staining for iron, T2 relaxometry, and MRI of labeled cell suspensions using FSE 3000/45 and GRE 300/20/20^0 sequences. Toxicity and proliferation of TA-Feridex labeled MSC was tested using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay (5).

Results: Figure 1 is an example of MSC and HeLa cells incubated with Feridex or MION-46L alone and with either Plus/Lipofectamine (PLFA) or Poly-l-lysine (PLL) as the TA. Without TA endosomal incorporation was very low for Feridex and undetectable for MION-46L after 48 hours (fig 1a,b,c) on PB staining. Numerous iron-containing intracytoplasmatic vesicles could be observed on PB stain Fig (1d,e,f) for the combinations of TAs with Feridex or MION-46l at 25 micrograms of Fe/ml. Cellular viability and proliferation of MSC was unaffected following labeling with either TA-SPIO or TA-USPIO. As compared to unlabeled (U) control cell suspensions, there was a dramatic increase in the 1/T2 values of TA-Feridex labeled (L) cells: ML T-cells (2X10^7 cells/ml) U = 0.87 and L = 3.7 s^{-1}; CG-4 (2X10^6 cells/ml) U = 1.9 and L= 23.3 s^{-1}; HeLa (2X10^7 cells/ml) U = 6.3 and L= 102.5 s^{-1}; and MSC (5x10^6 cells/ml) U = 2.7 to L=.80.6 s^{-1}at tau = 5 msec. Figure 2 contains the signal intensity (SI) measurements from MR imaging U and TA-Feridex L cells in gel with a 50-99% decrease in SI observed when comparing TA-SPIO to U cells on T2* GRE images.

Conclusions: We have demonstrated the ability to magnetically label SC and mammalian cells from various species efficiently by simply incubating Feridex or MION-46L nanoparticles with commercially available TAs. Macromolecular TAs probably binds to the (U)SPIO nanoparticle via electrostatic charges, coating the particle and thereby facilitating the transmembrane transport and incorporation into endosomes. This approach will allow for a universal, non-specific method of magnetically labeling of SC stem cells and provides researchers with the ability to track the extent of migration either following direct implantation or systemic injection in vivo using MRI.

References 1. Bulte JWM, et al. PNAS 1999;96:15256-15261.2. Josephson, L., et al Bioconj. Chem. 1999;10: 186-191. 3. Pouton, C.W et al. Adv Drug Delivery Rev 1998;34:3-20. 4. Strable, E., et al. Chem. Mater. 2001;13: 2201-2209. 5. Bulte, JWM et al Nature Biotechnology 2001;19:1141-1147.

Fig 1. PB staining (a,) Feridex+ MSC (b) Feridex+ HeLa,(c) MION+HeLa, (d) PLL+Feridex+MSC, (e) PLL+Feridex+HeLa, (f) PLL+MION+HeLa.

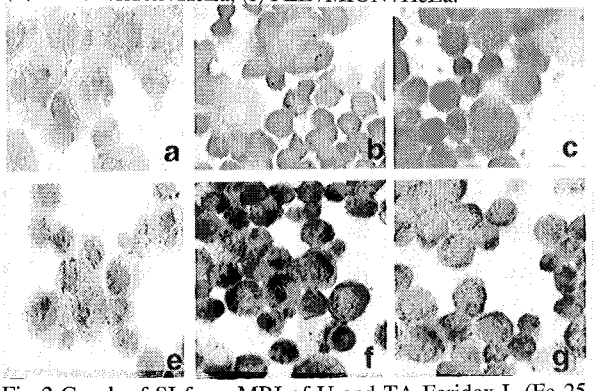

Fig 2 Graph of SI from MRI of U and TA-Feridex L (Fe 25 μg/ml) for ML, CG-4, HeLa, and MSC.cell suspensions in gel.