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MAGNETIC LABELING AND TRACKING OF CELLS USING MAGNETODENDRIMERS AS MR CONTRAST AGENT

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INTRODUCTION: During the last few years, the therapeutic use of stem and progenitor cells as a substitute for malfunctioning endogenous cells has received much attention. Unlike their use in animal models, the introduction of therapeutic cells in patients will require techniques that can monitor their tissue biodistribution non-invasively. Among the different imaging modalities, magnetic resonance (MR) imaging offers both near-cellular (i.e. 50 μm) resolution and whole-body imaging capability. In order to be visualized, cells must be labeled with an intracellular marker that can be detected by MR imaging. Superparamagnetic iron oxide nanoparticles provide currently the highest sensitivity when used as MR contrast agent. Following their covalent linking to anti-transferrin receptor internalizing monoclonal antibodies [1] or small HIV-tat peptides [2], they can be used as cellular contrast agent allowing MR tracking of magnetically labeled progenitor cells following transplantation. We have recently developed a new type of iron oxide nanoparticle, magnetodendrimers, that has excellent magnetic and NMR relaxation enhancing properties [3] and, due to its coating with a dendrimer as transfection agent, is effciently taken up by a variety of mammalian cells [4].

METHODS: Magnetodendrimers (MD-100) were synthesized with a stochiometric ratio of 100:1 of Fe:dendrimer as described [3]. Transmission electron microscopy revealed an oligocrystalline structure of 7-8 nm crystals separated by a somewhat smaller distance (see Figure 1).

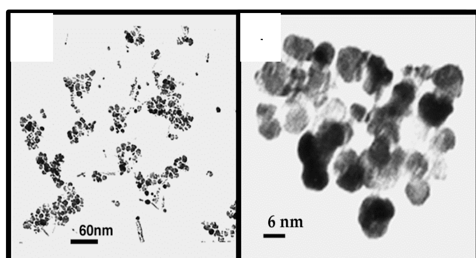

Fig. 1: TEM results for magnetodendrimer oligomers from synthesis with 100:1 Fe:dendrimer stochiometry (MD-100). The higher magnification on the right shows a cluster with individual iron oxide crystals.

MD-100 has a high saturation magnetization of 94 emu g/Fe, no magnetic hysteresis at 300 K, and exhibits T2 relaxivities of 200 $mM^{-1}s^{-1}$ (at 37 °C), with a rapid approach to saturation at magnetic field strengths well below 1.5 Tesla.

Cells were magnetically labeled by simply adding MD-100 to the culture medium at concentrations of 10-25 μg Fe/ml, and incubation of 1-2 days. This included mouse 3T3 fibroblasts, mouse C2C12 muscle progenitor cells, rat CG-4 oligodendrocyte progenitors, rat neural stem cell (NSC)-derived oligodendroglial progenitors, human HeLa cervix carcinoma cells, human GLC-28 small cell lung carcinoma cells, human endothelial progenitor cells, human NSCs, and human mesenchymal stem cells. Approximately 5 x 10^4 MD-100 labeled NSC-derived rat oligodendroglial progenitors were transplanted into the ventricles of neonatal (P=0) Long Evans shaker (*les*) rats. Cells were co-transfected with the LacZ gene (encoding for the enzyme β-galactosidase), in order to track them histochemically by incubating with the X-gal enxyme substrate. Animals were imaged on a weekly to biweekly basis using a clinical 1.5 Tesla clinical imager and a 4.7 Tesla animal imaging system.

RESULTS AND DISCUSSION: Prussian blue staining of magnetically tagged cells showed a remarkably high degree of intracellular labeling, with the cytoplasm containing large numbers of iron-containing vesicles or endosomes (Figure 2).

Achievement of intracellular labeling is a critical requirement, since a membrane-bound magnetic probe is likely to interfere with cell-tissue interactions (membrane recognition processes), may detach easily from the cell membrane, or may be taken up and transferred to other cells *in vivo*. All cells showed a comparable degree of uptake, demonstrating that the MD-100 uptake is non-specific and not dependent on the cell type or species.

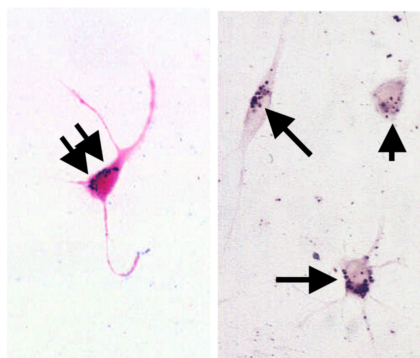

Fig. 2: Non-enhanced (left) and DAB-enhanced (right) Prussian Blue stains of MD-100 labeled, NSC-derived oligodendroglial precursor cells show the presence of numerous iron-containing vesicles in the cytoplasm.

The total iron content of cells labeled with 25 µg Fe/ml MD-100 for 2 days is approximately 10 pg Fe/cell, whereas unlabeled cells contained about 1 pg Fe endogenous iron. Magnetic labeling with MD-100 appeared irreversible, that is, the label is not excreted from the cells *in vitro* for at least 1 week following labeling. Labeled cells appeared unaffected in their proliferation capacity and viability, and an MTT-based toxicity assay showed no difference between unlabeled cells and MD-100 labeled cells. Furthermore, MD-100 labeled human NSCs showed comparable formation of neuronal processes when replated and grown for an additional 10 days, and differentiated normally into glial cells and neurons.

Following transplantation, migration of labeled cells into the brain parenchyma could be observed at the earliest time points (between 2 and 3 weeks) throughout the latest time points of imaging (6 weeks following transplantation). There was a good gross anatomical correlation with the macroscopic distribution of β-galactosidase-expressing cells. Moreover, the transplanted and labeled cells were also found to be able to form myelin that overlapped with the area of MR contrast. Figure 3 demonstrates an example of a 3D-reconstructed MR image obtained *in vivo* at the latest time point of imaging (6 weeks). While this image was obtained at high resolution using a dedicated high field (4.7 Tesla) animal scanner, the labeled cells could also be readily identified *in vivo* using a conventional 1.5 Tesla clinical imaging system. A comparison of several pulse sequences demonstrated that T2* weighted gradient echo imaging, that does not compensate for the induced dephasing of protons, was most sensitive to the presence of labeled cells. From the number of cells injected, and the overall area of contrast, we estimate that it should be possible to detect only a few cells when using T2* weighted imaging techniques.

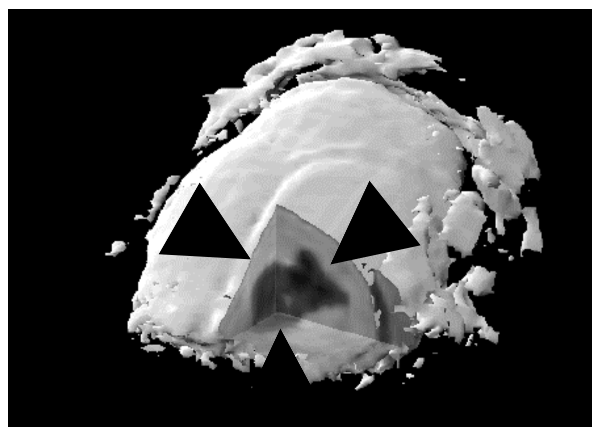

Fig. 3: 3-D Reconstructed in vivo MR image of les rat brain showing distribution of magnetically tagged oligodendroglial progenitors at 6 weeks following transplantation. Note the migration of cells into the parenchyma away from the ventricle (arrowheads).

CONCLUSIONS: Magnetodendrimers represent a new class of cellular MR contrast agents that can be used for a high degree of magnetic cellular labeling regardless of animal species. Following transplantation of MD-100 labeled oligodendrocyte progenitors, cells can be followed in vivo for at least 6 weeks following transplantation, with a good histopathologic correlation including the formation of myelin. The prospect of MR tracking of labeled cells appears attractive not only as a tool to perform longitudinal cell migration studies in the same animal, but also because of its potential to help guide future clinical studies involving the use of therapeutic stem and progenitor cells.

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