Longitudinal Detection of Neuronal Stem Cells Labeled with Two Types of Iron Oxide Particles

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Introduction: Magnetic resonance imaging (MRI) provides an effective way to monitor implanted stem cells non-invasively [1]. Detection of implanted cells depends primarily on the type and concentration of the contrast agent used for labeling. Due to cell division, migration, and metabolism, signal loss is generally observed. It is thus important to perform an *in vivo* longitudinal study to evaluate the extent of cells detection (in time) following transplantation. In this study we compared two types of contrast agents: the nanometer size iron oxide particles (SPIO) and the micron size iron oxide particles (MPIO) for long-term detection of labeled stem cells. Labeled stem cells (C17.2) were neonatally transplanted into the normal mouse brain and *in vivo* MR imaging was performed serially for four months.

Methods: *Cell culture*: C17.2 cells were maintained on uncoated T75 flasks in DMEM with 10% FBS and 5% horse serum. *Labeling of cells with iron oxide particles:* C17.2 cells were incubated with SPIO (100 μg Fe/ml, (Feridex, Berlex labs, NJ)) and MPIO ($1.28x10^8$ particles, 0.96 μm-size (Bangs Laboratories, IN)) for 24 hours as described earlier [2, 3]. These concentrations lead to a net iron concentration of ~ 46 pg/cell. *Intra-cranial implantation:* Neonatal C3H/SCID (n=12) mice were cryo-anaesthetized and injected on the day of birth. Two μl of the labeled C17.2 cell suspension ($5x10^4$ cells/μL) were injected into each lateral ventricle and the animals were imaged 1, 2, 3 and 4 months after implantation. *Imaging:* 3D gradient-echo images were acquired on a 4.7 T magnet using a 2.5 cm birdcage coil. In order to track the signal changes over time, the images acquired at different time points were spatially aligned with each other. Briefly, one data set was selected as the reference and all the subsequent images were rigidly aligned using an automatic registration algorithm, called FLIRT [4].

Results: Both SPIO and MPIO labeled cells were detected *in vivo* during the four-month period and no significant difference in detection of implanted cells labeled either with MPIO or SPIO particles were detected during this period (Figure 1). Comparison of MR images acquired 1, 2, 3, and 4 months after implantation revealed the presence of three types of hypo-intense signals. The first type of signal remains un-changed over time (Figure 2, white circle), the second type of signal became smaller over time (green circle) and the third type of signal expanded during the first two months and reduced in the subsequent two months (red circle).

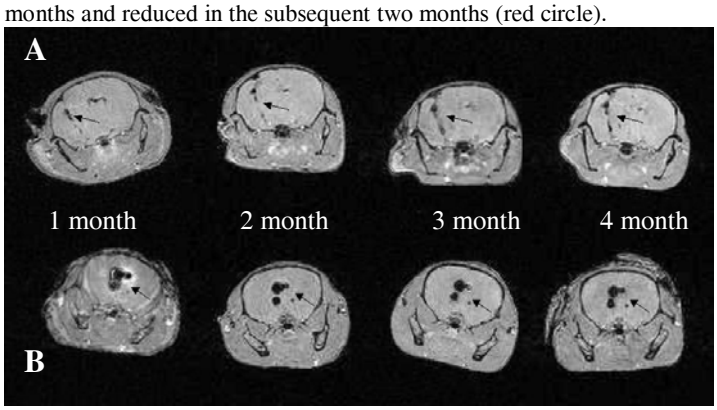

Figure.1 Representative transverse sections from 3D gradient echo images of a mouse brain 1, 2, 3 and 4 months after neonatal implantation of (A) MPIO and (B) SPIO labeled C17.2 cells. Imaging parameters: TR/TE = 100/4.5 ms, matrix = 128x128x128, FOV = 2.0 cm, nt = 4, acquisition time ~ 100 min, resolution ~ 156 μm. Arrows point to the areas of hypointense signals due to the presence of labeled cells. Cells labeled either with MPIO or SPIO iron oxide particles could be detected for four months after transplantation.

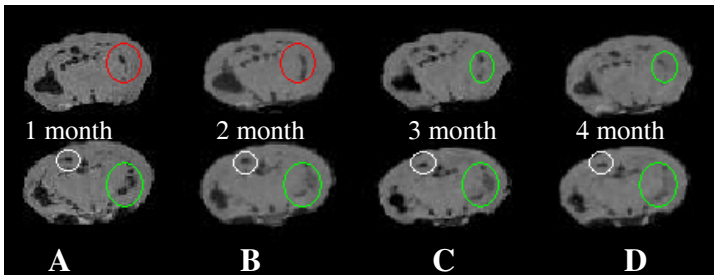

Figure.2 Co-registered transverse sections from 3D gradient echo images of a mouse brain 1 (A), 2 (B), 3 (C) and 4 (D) month after neonatal implantation of SPIO labeled C17.2 cells. Imaging parameters: TR/TE = 100/4.5 ms, matrix = 128x128x128, FOV = 2.0 cm, nt = 4, acquisition time ~ 100 min, resolution ~ 156 μm. Three types of hypo-intensity signals were observed. The first: the signal remains un-changed over time (white circle), the second: the signal became smaller over time (green circle) and third type of signal expanded in time (red circle).

Discussion and Conclusion: Non-invasive tracking of grafted cells opens the possibility to follow the migration pattern of implanted stem cells *in vivo*. Our studies demonstrate that neuronal C17.2 stem cells labeled with SPIO or MPIO iron oxide particles can be detected by *in vivo* MRI indicating that the smaller sized SPIO particles are as good as the larger sized MPIO particles for *in vivo* detection up to four months after transplantation. We observed three patterns of changes in the hypo-intense signal from labeled cells. In the first category, the hypo-intensity remained at the same location and intensity during the observation period. This type of signal probably reflects the presence of engrafted cells that were not migrating after the initial relocation. The second pattern included hypo-intense signals that gradually disappeared reflecting the dilution of contrast agent probably due to division of the grafted cells. The most notable feature was the third type of signal with some hypo-intense areas appearing in the region that were not there originally (red circle). This type of signal indicates that grafted stem cells continue to migrate in the normal mouse brain for at least two months after the implantation. Migration of grafted stem cells toward a lesion have been reported earlier [5]. In this study we demonstrated that migration of implanted stem cells takes place in a normal mouse brain up 2 month after implantation of C17.2 cells neonatally. Although preliminary, these results could aid in development of optimal strategies for stem cell based therapies.

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Reference: 1) Allport, J.R., Experimental Hematology, 2001, **29**: p.1237-46 2) Magnitsky, S., NeuroImage, 2005, **26**: p. 744-754 3) Shapiro, E.M., PNAS 2004, **101**: p. 10901-6. 4) Jenkinson, M., Neuroimage, 2002, **17**: p. 825-41 5) Hoehn, M., PNAS 2002. **99**: p. 16267-72.