Cell Motility of Neural Stem Cells is Reduced after SPIO-Labeling which is Mitigated after Exocytosis

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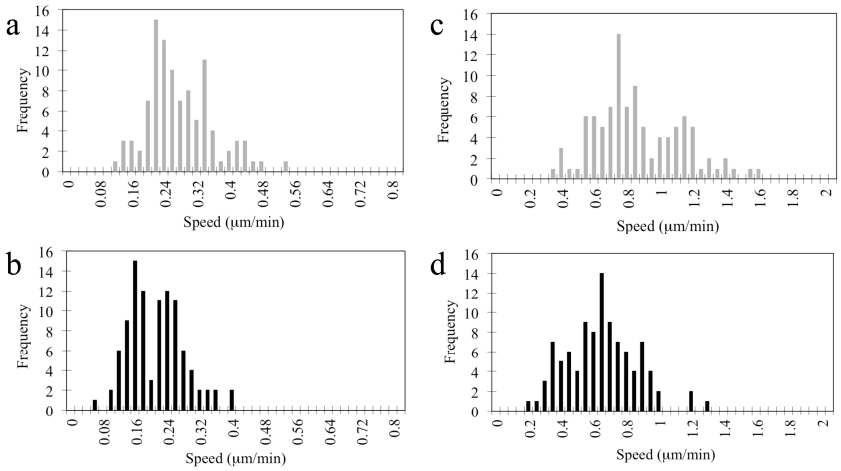
Introduction: Clinical MRI cell tracking has recently emerged as a predominant imaging modality, in particular for neural stem cell (NSC) tracking in the the CNS (1-3). The primary clinical application of MR tracking of NSCs is likely to be monitoring delivery of cells in real-time, using MR-compatible catheters, with visualization of their initial engraftment and homing shortly after administration. Long-term tracking of NSCs will be challenging, as it is difficult to discriminate live from dead cells, and rapidly dividing NSCs may exhibit a pronounced mismatch between the MRI and histological distribution pattern soon after their engraftment (4). It has been suggested that a primary reason for this MRI mismatch, i.e., cells becoming undetectable, is their cell division and associated dilution of label. However, to the best of our knowledge, there have been no studies on a possible direct effect of SPIO-labeling on NSC motility and migration. We SPIO-labeled murine NSCs and assessed them for in vitro for cell proliferation, cell division, and random motility using time lapse video microscopy. In addition, labeled cells were transplanted into immunodeficient mice and followed *in vivo* for 4-72 hours.

Materials and Methods: Immortalized *LacZ*-transfected C17.2 cells were constitutively transduced with firefly luciferase and labeled with Molday ION Rhodamine B and CellTracker Green CMFDA. Cells were cultured and observed in either normal culture medium or differentiation medium (at 2, 4, 6, or 8 days of differentiation prior to starting microscopy). Phase-contrast and fluorescent (green and red channels) images of live cells were taken using a cascade 512BII CCD camera. Images were taken every 30 minutes for 24 hours for each experiment. Live cell microscopy experiments were analyzed to locate cellular division events; over 150 division events were monitored. Using a custom-designed program in Matlab 7.9, a region of interest (ROI) was manually drawn around cells presented in a graphical interface using either the green (CellTracker Green) fluorescent image or the phase-contrast image. The ROI was projected onto the corresponding red (Molday ION Rhodamine B) fluorescent image and the red fluorescence in each parent cell and daughter cells during a cell division event was quantified. For determination of cell motility, images were taken every 5 minutes for 12 hours. In all experiments, cell images were visually examined before analysis; cells displaying morphologic changes characteristic of apoptosis were excluded. A Time Lapse Analyzer cell tracker analysis program was used to quantitatively track cell motility. The following parameters were evaluated and statistically compared: 1) The medium (average), 2) the median, and 3) the maximum speed for each cell cohort, Statistical analysis was performed using a Student's t- and ANalysis Of VAriance between groups (ANOVA) test. $2x10^5$ cells were injected into immunodeficient Rag2 mice (n=3 per time point). Bioluminescence imaging (BLI) was performed immediately and every 24 hours for 3 days after transplantation. Fluorescent immunhistology was used to validate the *in vivo* findings.

Results and Discussion: Fig. 1 shows histograms with the distribution of (a) the average speed of unlabeled C17.2 cells, (b) the average speed of SPIO-labeled cells, (c) the maximum speed of unlabeled C17.2 cells, and (d) the maximum speed of SPIO-labeled cells. The average speed for unlabeled and SPIO-labeled cells was 0.27 ± 0.04 and 0.20 ± 0.03 μm/min, respectively (95% confidence interval). The average of the maximum speed was 0.84 μm/min for unlabeled cells and 0.63 μm/min for Molday ION labeled cells. Unlabeled cells outperformed the labeled cells in terms of motility for all *in vitro* measured parameters by a ratio of 1.3:1. At 4 and 24 hours post engraftment, there was a near 100% co-localization between cells and SPIO label. Starting at 48 hours, C17.2 cells were found to exocytose the label, and cells that were devoid of SPIO were those migrating farthest away from the site of transplantation. With nearly 100% of cells labeled and a doubling time of approximately 20 hours, the intracellular clearance of SPIO-label at 48 hours cannot be explained by label dilution alone, as on average only 2.5 cell divisions are occurring. Rather, an explanation for this rapid loss could be early cell death and extracellular, passive release of SPIO. However, BLI demonstrated that cell death *in vivo* following transplantation was negligible. In addition, TUNEL, anti-PCNA, and caspase-3 immunostaining demonstrated that labeled cells did not undergo apoptosis. Based on these observations, the only explanation for the rapid loss of SPIO is active exocytosis. Interestingly, we found that cells that migrated away from the injection site were those devoid of label. Our results are in agreement with other recent reports have shown that SPIO-labeling can affect the actin cytoskeleton and microtubule architecture (the machinery of cell motion) of labeled cells in a dose-dependent manner, possibly due to sterical hindrance of SPIO-containing endosomes with the actin fiber network (5,6).



Conclusions: We have shown here that SPIO-labeling can reduce the overall motility of transplanted cells, both *in vitro* and *in vivo*, but that exocytosis of SPIO mitigates this adverse effect making this only a temporary phenomenon. Therefore, the use of MRI cell tracking should stay limited to guide real-time MR guided injections and to only monitor the initial engraftment.

References: (1) D. Karussis et al., Arch. Neurol. 67:1187-1194 (2010). **(2)** J. Zhu et al., N. Engl. J. Med. 355:2376-2378 (2006). **(3)** S. Jozwiak et al., Cell. Med. Part B Cell. Transpl. 1:71-80 (2010). **(4)** P. Walczak et al., Magn. Reson. Med. 58: 261-269 (2007). **(5)** A. Khaleghian et al., Afr. J. Biochem. Res. 4:99-104 (2010). **(6)** S.J. Soenen et al., Small 6:832-842 (2010).