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Enhanced MRI visualization of endogenous neuroblasts migration by optimizing MPIO formulations

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INTRODUCTION: *In vivo* labeling of endogenous neural progenitor cells in adult rat brains is an established method for tracking native cell migration in vivo. Micron sized iron oxide particles (MPIOs) are injected into the lateral ventricle proximal to the neural stem cell niche in the brain and are endocytosed by neural progenitor cells, making them visible by susceptibility weighted gradient echo MRI. However, the in vivo efficiency of MPIO uptake into stem and progenitor cells remains very low, with only 5-17% of the NSC harboring the MPIOs (Sumner, et al *Neuroimage* 2009). Such low labeling efficiency hampers progress in using MRI based single cell tracking in brain pathologies. Recent attempts to improve in vivo cell labeling in mice include direct injection of particles into the RMS (Neiman, et al *Neuroimage* 2010) and a combination of MPIO and poly-L-lys (PLL) (Vreys, et al *Neuroimage* 2009). We have previously demonstrated that enhanced MPIO cell labeling can be achieved within 2 hours of incubation in labeling media containing a mixture of 1.63 µm MPIOs and transfection agent (Tang, et al *ISMRM* 2010). Here we extend this paradigm to improve the in vivo uptake of MPIOs in rat brains and enhance in vivo MRI detection of migrating precursor stem cells.

MATERIALS AND METHODS:

In vivo labeling: Intraventricular injections of MPIOs into rat brains were performed as described (Shapiro et.al 2006). We assessed various modifications of the currently used MPIOs to improve endogenous labeling (Table 1). Modifications included the addition of transfection agents, PLL and protamine sulfate (PS); the use of a positively charged as well as reduced size (0.86µm versus 1.63µm). PLL and PS concentrations were based on well established literature (Frank, et al *Radiology* 2003, Arbab, et al *Transplantation* 2003). Equivalent volumes and amount of iron were delivered with all variations tested. 3D gradient echo images were acquired at 1 and 14 days post injection, at 11.7T with the following parameters: TE = 8 ms, TR = 30 ms, 100 µm^3 resolution.

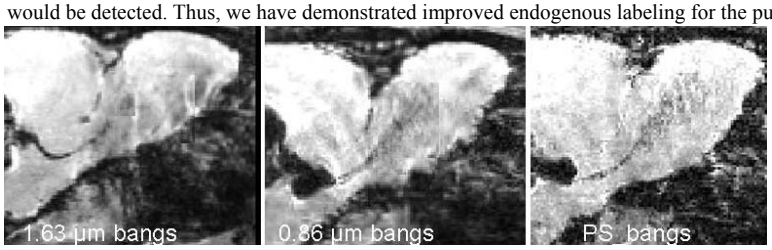
group	n	Size (µm)	coat	charge	complex	incubation
a	5	1.63	carboxyl	negative	none	none
b	4	1.63	carboxyl	negative	PLL 1nM	Mix and immediate injection
c	4	1.63	carboxyl	negative	PS 1.25µM	Mix, 15' incubation, injection
d	4	1.43	amine	positive	none	none
e	4	0.86	carboxyl	negative	none	none

* All particles were purchased from Bangs laboratories coated with styrene/Divinyl benzene. All injections were 20 µl of 66 µg Fe.

Data analysis: 3D data sets were used to quantify the volume of dark spots in the two olfactory bulbs at 14 days post injection. Datasets were processed using BioImage Suite. First a rigid linear registration was applied to all brains according to a predetermined template. Neuronal precursor cell migration from the SVZ, along the RMS to the OB was quantified by detection of dark signal voids in the OB. We have defined pixels containing labeled cells by applying a threshold of > 30% decrease in signal intensity relative to the signal of non-enhanced tissue. The occupancy of labeled precursor cells in the two OBs (in mm^3 volume), was normalized to the volume of the OBs. The volume percentage of dark pixels within the OBs in rats injected with standard 1.63µm MPIOs was set as a reference for the efficiency of endogenous stem/progenitor cell labeling. Quantified dark volumes in the OBs following injection of modified MPIOs were normalized relative to standard MPIOs.

RESULTS and DISCUSSION

Accumulation of labeled cells in the OBs was assessed at 14 days post injection and was determined as the percentage of volume occupancy of dark pixels in the OBs. Increased fractions of dark volume within the OBs relative to currently applied 1.63µm MPIOs was observed for all modified MPIOs tested in this study. Supplementing standard MPIOs with 1nM PLL increased the efficiency by 20% and using an amine modified MPIO enhanced the efficiency by 48% (Figure 1). These elevations however were not statistically significant (p=0.67 and p=0.22, respectively). Considerable and statistically significant improvements in labeling efficiencies were observed for both PS supplementations as well as for labeling with smaller particles of 0.86µm. These elevations were of 168% (p=0.03) and 428% (p=0.04), respectively. These enhanced monitoring of cell migration is evident in the presented MR images (Figure 1, left). The fraction of the dark volume within the OBs corresponds to labeled migrating precursor cells. The more efficient initial labeling, the more signal accumulates in the OBs. This could result from either increased MPIOs per cell or increased labeled cell numbers. Either way, enhanced labeling of the stem cell niche would lead to increased numbers of labeled migrating cells that would be detected. Thus, we have demonstrated improved endogenous labeling for the purpose of MRI detection of cell migration.



	1.63 µm bangs	1.63 µm, PS	1.63 µm, PLL	1.41 µmNH2	0.86µm
# of rats	5	4	4	4	4
average	**0.51**	**1.38**	**0.61**	**0.76**	**2.71**
efficiency	100.00	268.34	119.48	148.43	528.65
se	15.78	23.86	16.18	9.31	65.00
	t-test	0.03	0.67	0.22	0.04

** average is the % volume (mm^3) of detected spots per two olfactory bulbs
** efficiency is % compared to 1.63 µm bangs

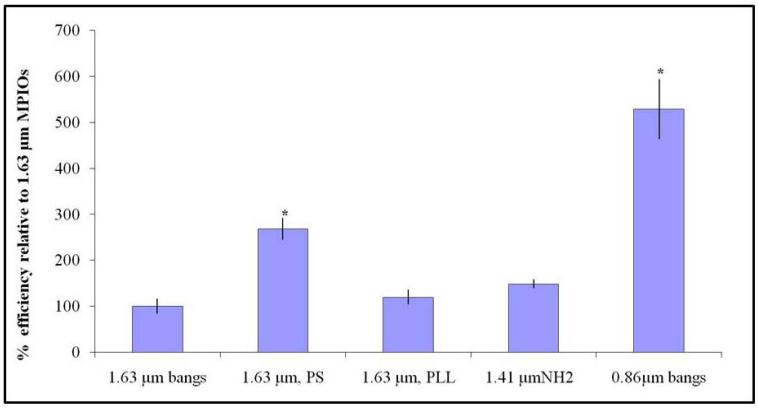


Figure 1: Improved detection of migrating labeled cells in the OB. Upper left, slices of 3D MRI datasets showing the degree of labeled precursor cells migration along the RMS to the OB. Shown are 1.63µm (left); 0.86µm (center) and 1.63 µm with PS (right). Labeled, migrating cells are detected as dark contrast moving from left to right. Upper right, a table with a summary of the volume occupancy and relative efficiencies obtained for the modified MPIOS. Left, graphical presentation of the results.

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