



the cellular survival. These potential drawbacks can be overcome using the most commonly used class of clinical MRI agents based on paramagnetic Gd-based complexes. These chemicals produce bright spots on T<sub>1</sub>-weighted MRI and include molecules provided with extremely high thermodynamic and kinetic stability, hydrophilic character, small size, and biological inertness. Nevertheless, clinically-approved Gd-based agents cannot compete with IONs in terms of contrast detection sensitivity, and they still represent a challenging implementation in the field of imaging MSCs graft.

An important step forward to bridge the gap between Gd-based agents and IONs for *in vivo* cellular imaging has been recently achieved, allowing the cytosolic entrapment of high amount of the clinically approved MRI agent Gadoteridol (marketed as ProHance™) through a novel cell labelling procedure based on osmotic shock [6]. The escape from the endocytic internalization pathway is very crucial for enhancing the sensitivity in the detection of the bright contrast because the endosomal entrapment of the labelling agent is inevitably accompanied by a “quenching” of the T<sub>1</sub> contrast [6]. Based on our experience, we argued that this labelling procedure could be helpful in tracking the migration of “therapeutic” MSCs in a murine SCI model (hemisection) [7]. Indeed, in addition to ascertain improvements in both cellular uptake and sensitivity in contrast detection *in vitro*, we found that the monitoring of MSCs migratory dynamics *in vivo* was feasible for about 10 days. By implanting MSCs about 1 mm caudally to spinal lesion site (as shown in the Figure below), it was possible to observe a migratory stream of cells progressively reaching the injured area in about 7 days. Moreover, the absence of alterations in the biological and functional profile of MSCs after the labeling step was verified *in vitro*, and confirmed *in vivo* by the evident motor recovery shown by treated animals, thus indicating the unaltered therapeutic efficacy of cells. In the context of experimental research, this efficient, safe, reliable, and simple labeling technique may serve to (i) test and compare different transplantation protocols, (ii) dynamically assess cell distribution, (iii) correlate the beneficial effects of the cell graft (estimated by behavioral tests) to the MRI observations, and (iv) analyze the ability of the injected cells to move in response to chemotactic stimuli *in vivo* and in real-time. Due to the relatively limited persistence of the contrast, the translational significance of such technique appears restricted to the early assessment of cell survival during the first days after injection, whose relevance however should not be underestimated considering that the detrimental response of the host CNS to the graft can determine death of exogenous cells within the first hours after transplantation [8].

In conclusion, the hypotonic labeling with positive MRI CAs represents a significant technical improvement in preclinical imaging of MSCs as applied to murine SCI, being potentially valid also in larger animals and humans, and lays the basis to address the current challenges imposed by the complexity of the CNS and the specific medical requirements of the stem cell-based therapies.

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**Keywords:** cellular imaging, mesenchymal stem cells, regenerative medicine, spinal cord injury, MRI, Neuroscience

**Received:** January 03, 2017

**Published:** January 13, 2017

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