Monitoring of Iron-PLLA Particle Loaded MSCs after Intramuscular Injection in the Rat Model @ 3T

V. Rasche[1], N. Fekete[2], A. Bornstedt[1], J. Zhu[3], I. Vernikouskaya[3], M. Urban[4], K. Landfester[4], G. Schmidtke-Schrezenmeier[2], and H. Schrezenmeier[2]
[1]Internal Medicine II, University Hospital Ulm, Ulm, Germany, [2]Institute for Transfusion Medicine, University Hospital Ulm, [3]Internal Medicine II, University Hospital Ulm, [4]Max-Planck-Institute for Polymer Research

Background: Cell therapies are a very active field of research. For proper identification of the optimal application mode ensuring efficient deposition and sufficient continuance of the cells at the target position, a detailed understanding of the cell trafficking and homing is required. Iron-labelled cells have been proven to enable in-vivo monitoring of the trafficking and homing of cells after injection with high sensitivities, which even enabled the visualization of a single cell (1). To ensure efficient cell labelling, sufficient uptake and trapping of the MR contrast agents in the cells must be obtained without altering its properties. Recently the use of Poly-L-Lactic Acid (PLLA) iron loaded nanoparticles for MSC labeling was suggested (2). It was shown that Iron-PLLA particles are well taken up by MSCs without alteration of properties of the MSC. Furthermore, they show high ionic relaxivity with r2* values as high as 600 $mM^{-1} \cdot s^{-1}$ and a very efficient uptake of the particles by the cells. It is the objective of this study to investigate the feasibility of iron-PLLA particles (iPLLA_P) for in-vivo monitoring of labelled MSCs after injection in a rat model.

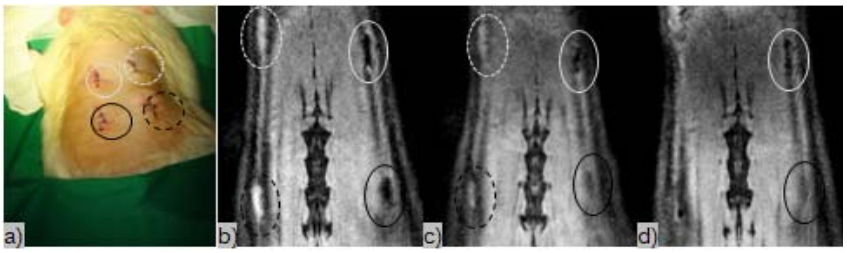

Fig1: Cells embedded in collagen pads: a) implantation sides; b-d) MRI 2d, 15d, and 21d after implantation (0.3M labelled cells / ml (solid black), 0.9M labelled cells / ml (solid white), 0.9M cells / ml (dashed black), collagen pad (dashed white))

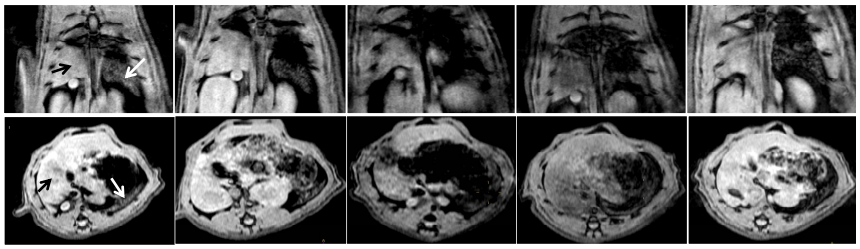
Fig 2: Signal changes in the liver (black arrow) and spleen (white arrow) in coronal and axial view orientation (lr: 15mn, 60mn, 8h, 5d, 9d after injection)

Methods and Materials: For initial investigation of the in vivo visibility of the iPLLA_P-labelled MSCs, collagen pads loaded with different concentrations of MSCs were implanted in a Wistar rat. For further assessment of the applicability of the particle for monitoring cell trafficking and homing, 2 Wistar rats were imaged before and after setting two cutaneous punch biopsies left and right to the spine. After punching, $5*10^5$ labelled MSCs were injected subcutaneously in the vicinity of the right punch lesion and a second dose was injected into paravertebral muscles on the left side. MR imaging was performed before, 15min, 60min, 8h, 5d and 9d after injection. The MRI imaging protocol comprised a three-dimensional steady-state gradient echo technique. Fat suppression was obtained applying a 11 spectral selective binominal excitation pulse. Image parameter were as: flip angle 15°, spatial resolution 300x300x400µm, TE/TR = 7.1/14ms, acquisition time 14min.

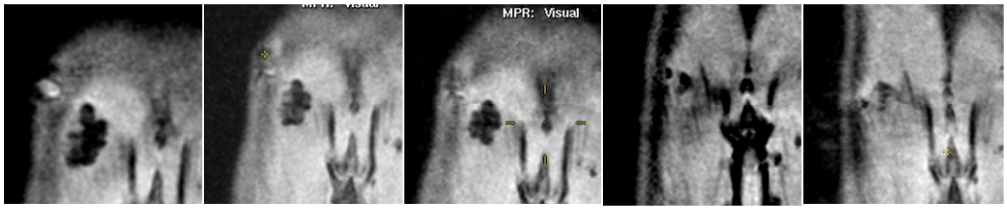

Fig3: Monitoring of the iron-PLLA particle labeled MSCs (lr: 15mn,60mn,8h,5d,9d after injection)

Results: A clear signal reduction was observed at the location of the pads containing iPLLA_P-labelled cells. The signal reduction was maintained for 21days with continuously decreasing amplitude (fig1). In the injury model, after injection of the iPLLA_P-labelled MSCs, local signal alteration was observed over the 9day follow-up period (fig3). Starting 8h after injection, some increasing signal voids were observed in the vicinity of the lesion. No significant signal alterations were observed in the liver, whereas substantial signal reduction was observed in the spleen (fig2).

Discussion: Iron-loaded PLLA particle can be used for efficient labelling of MSCs. Signal alteration can be maintained in vivo for at least 9 days after intramuscular injection of labelled MSC or after injection of labelled cells around a cutaneous wound. The sole signal reduction in the spleen indicates accumulation of iron in the spleen. Since it has been shown earlier (3) that circulating MSCs can home in the spleen, the signal reduction in the spleen is an indicator for the presence of iron-loaded MSC in the spleen. This needs to be confirmed by demonstration of the presence of iron-loaded human MSC with different methods.

References: (1) Shapiro EM, et al. PNAS 2004;30:10901-10906. (2) Schmidtke-Schrezenmeier G., et al. ISMRM 2010. (3) Rüster et al. BLOOD 2006, 108; 3939-3944