Monitoring of Iron-PLLA Loaded MSCs after Silicone Carrier Application @ 11.7T

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BACKGROUND: As previously shown (1) the labeling of the mesenchymal stem/stromal cells (MSCs) with Poly-L-Lactic Acid (PLLA) iron loaded nanoparticles (iPLLA) does not affect cell biology, e.g. cell proliferation, differentiation capability, clonogenicity and adhesive properties compared to unlabeled controls. It was also previously shown (2) that the signal alteration due to administering of the labeled MSCs per intramuscular injection in vivo can be observed for at least 9 days after injection. The attachment and proliferation of the donor MSCs on the medical grade silicone surface with a plasma-polymerized acrylic acid coating (silicone carrier) was proven by a group of the University of Sheffield (3, 4). Our objective was to use this chemically defined silicone carrier as an delivery aid of the iPLLA-labeled stem cells to the skin wounds for the further monitoring of the cell trafficking using MRI.

METHODS and MATERIALS: **Magnetic Properties of iPLLA nanoparticles**. The new synthesized iPLLA nanoparticles contain iron oxide as well as fluorescense dyes perylenmonoimide (PMI) and IR-780 iodide, allowing its application as triple reporter nanoparticles. For the description of magnetic properties of this iPLLA particles a phantom with different particle concentrations were prepared in 2% gelatin. The imaging was done at a 3T whole-body system (Achieva, Philips Medical System) and at 11.7T BioSpec animal system (Bruker BioSpin MRI). The T2/T2* quantification showed the r_2 relaxivities of iPLLA particles comparable to such of a Resovist®: r_2 (iPLLA) = 324.07mM^{-1}s^{-1} vs. r_2 (Resovist®) = 342.89mM^{-1}s^{-1} at 11.7T and r_2 (iPLLA) = 207.1mM^{-1}s^{-1} vs. r_2 (Resovist®) = 245.06mM^{-1}s^{-1} at 3T; r_{2*} (iPLLA) = 350.97mM^{-1}s^{-1} vs. r_{2*} (Resovist®) = 306.67mM^{-1}s^{-1} at 3T.

In Vitro Imaging. MSCs were collected from bone marrow aspirations (iliac crests) of volunteer healthy donors. Collection of this material has been approved by the Ethical Committee of University of Ulm and informed consent of patients and healthy donors has been obtained. Nanoparticles uptake by the cells was previously confirmed via flow cytometric analysis of PMI fluorescence. Cell toxicity was measured via 7-AAD labeling and found to be 0.5% compared to unlabeled cells. Analysis of labeled cells by scanning electron microscopy confirmed characteristic spindle-shaped fibroblast-like cell morphology and cell adhesion to the carrier material. iPLLA-labeled MSCs were adhered to 1mm thick silicone carrier with a surface of 2cm^2 with a cell seeding density = 39 215.7 MSCs/cm^2, corresponding to 20 000 cells per silicone carrier with 8mm diameter (~ 0.5 cm^2), which was also used for in vivo experiment later on. This 20 000 cells were found to be the optimal cell number for 8mm carrier surface, as higher numbers of cells were observed to detach in vitro from the carrier. The imaging of the carrier in 2% gelatine was done at 11.7T with 3D FLASH sequence and following acquisition parameters: FOV 3.5x3.5x0.5cm, matrix size 696x696x24 and corresponding resolution of 50x50x125µm, TR/TE = 50/5.6ms, flip angle 10°, bandwidth 50kHz and NEX 2.

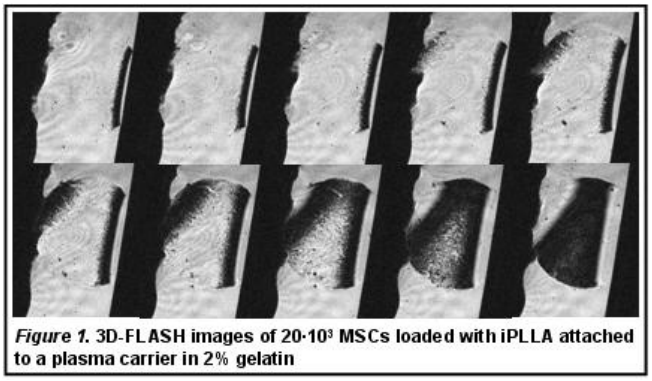

Figure 1. 3D-FLASH images of 20·10³ MSCs loaded with iPLLA attached to a plasma carrier in 2% gelatin

In Vivo Imaging. For in vivo MRI experiment (11.7T) a female Wistar rat was shaved in the lower dorsal area (Fig.2a). 8mm punch biopsy scalpels were used to create two defined skin wounds (Fig.2b). Silicone carriers with 20 000 iPLLA-labeled MSCs were then transferred "face down" onto these wounds: carrier with the labeled human MSCs on the right side and with the rat MSCs - on the left side of the body (Fig.2c). After 3h the carriers were removed (Fig.2d,e). The axial slices were obtained with 3D fat suppressed FLASH sequence and following imaging parameters: FOV 5x5x1.5cm, matrix size 500x500x30 and corresponding resolution of 100x100x500µm, TR/TE = 25/2.7ms, flip angle 6°, bandwidth 100kHz and NEX 2, acquired within 12.5min.

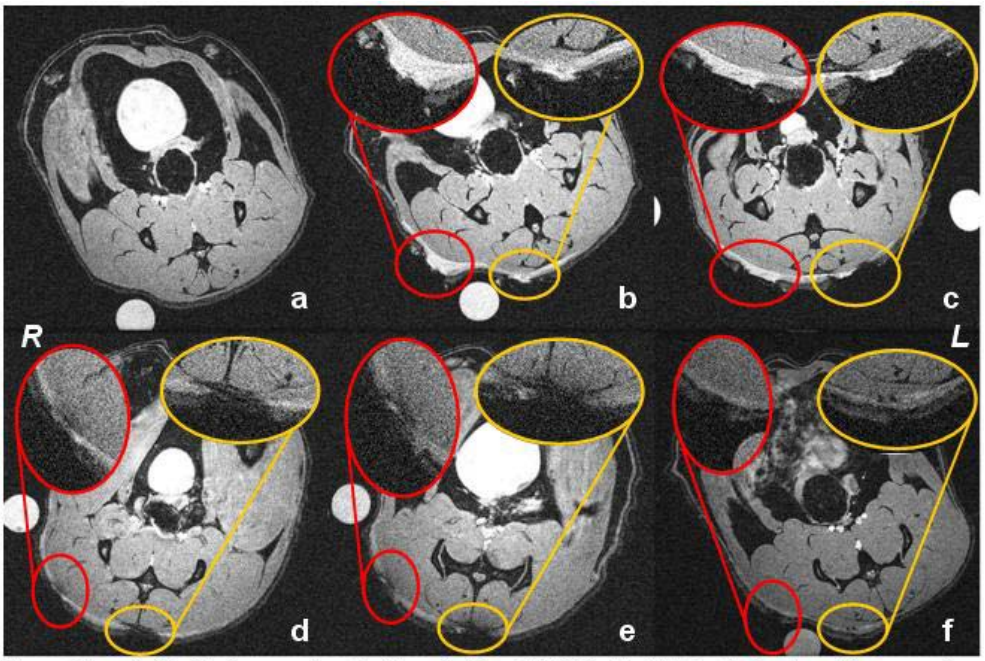

Figure 2. Longitudinal in vivo experiment with application of iPLLA-labeled MSCs attached to a silicone carrier (R – right hand side – human MSCs; L – left hand side – murine MSCs): a) reference scan, b) skin wounds, c) carriers application, d), e) two slices 3h after carriers application (carriers removed), f) 3d after carriers application

RESULTS: A characteristic iron artifacts could be perfectly observed on transversal slices of the carrier surface in vitro (Fig. 1). The in vivo signal voids appeared in the skin wound after carrier application (Fig.1c) could still be observed after carrier removing (Fig.1d,e), and even 3d later (Fig.1f). Please note that after 3h, a diffuse signal reduction can be appreciated indicating a migration of the MSCs into the surroundings of the wound. After 3d, the signal voids appear to be restricted to the wound area, indicating the attraction of the MSCs to the injury.

DISCUSSION: Silicone carriers appears as a feasible vehicle for the application of MSCs to surface wounds. In the animal model, the fixation of the carrier turned out to be difficult and more sophisticated fixations such as e.g. Teflon wound chambers may be required for keeping the carriers for a longer time (48h) on an in-life rat.

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