Poly-L-Lactic Acid (PLLA) Iron Loaded Nanoparticles for MRI Cell Labelling

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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. To ensure efficient cell labelling, sufficient uptake and trapping of the MR contrast agents in the cells must be obtained without altering its properties. The objective of this study was to investigate the feasibility of applying iron-loaded poly-L-lactic (PLLA) nanocapsules for cell labelling.

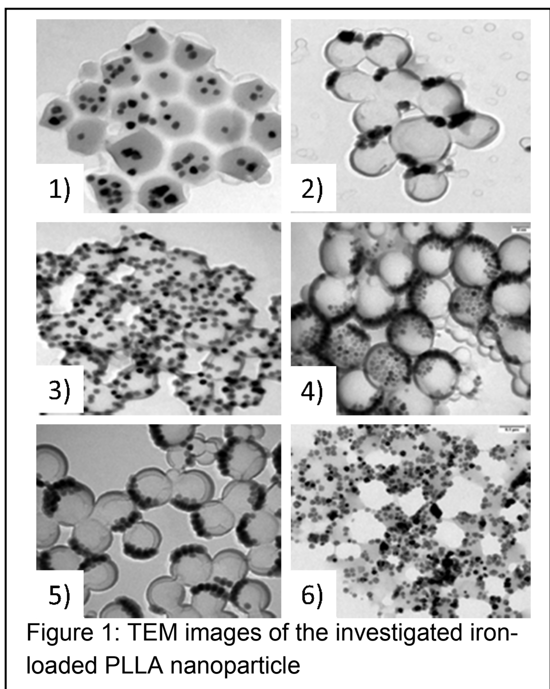

Figure 1: TEM images of the investigated iron-loaded PLLA nanoparticle

Methods and Materials: Six different iron-loaded (FeO) PLLA nanocapsules were synthesized by the mini-emulsion techniques [1]. The size of the particles ranged between 110-135nm with a zeta-potential of -28mV to -55mV and iron-loads between 0.4mg/ml to 2.73mg/ml (see Fig. 1). The iron content and distribution in the capsules varied as well as the diameter of the iron cores. The different nanocapsules were investigated regarding their MRI properties (T2 and T2* relaxivities in agarose gel), their cell uptake and persistence (TEM, flowcytometry, confocal laser scanning microscopy, and Prussian blue staining), toxicity (7-AAD staining), and alteration of the cell properties (osteocyte, adipocyte and chondroblast differentiation potential). Furthermore, the visibility of labelled MSCs embedded in agarose gel was monitored over a time period of 144h. All MRI measurements were performed at a 3T whole-body system (Achieva, Philips Medical Systems, Best, The Netherlands). T2 maps were obtained by using spin echo sequence (TR / ΔTE / Echoes / Flip angle = 1500ms / 25ms / 8 / 90º) and T2* maps were obtained by using gradient echo sequence (TR / ΔTE / Echoes / Flip angle = 500 ms / 3ms / 16 / 55º).

Particle	*Iron Load [mg/ml]*	*r2* [$mM^{-1}\cdot s^{-1}$]	*r2** [$mM^{-1}\cdot s^{-1}$]
1	0.48	243.6	**226.2**
2	1.0	46.25	**478.8**
3	2.35	35.78	**607.4**
4	2.57	27.04	**469.84**
5	2.68	28.39	**357.84**
6	2.73	22.4	**294**
Resovist®		**276.08**	**245.28**

Table I: Relaxivities of the different investigated particles.

Results: As expected, the ionic MRI T2 and T2* relaxivities of the different capsules widely varied with the load and distribution of the iron in the nanocapsules. By proper clustering and loading of the particles excellent ionic T2* relaxivities with only low T2 relaxivities could be achieved. Iron-labeled PLLA nanoparticles were taken up rapidly by MSCs. Intracellular particle persistence was verified up to 144 h after incubation. In MRI 96 h after particle removal 2×10^5 iron-labeled MSCs could be clearly discriminated from 5×10^4 cells and from unlabeled cells in an agarose phantom. Iron-PLLA particle labeled MSCs maintained their differentiation potential and showed an excellent viability. Standard surface markers were not influenced by the labeling. Differences were detected with regard to CD71. In the iron-PLLA particle labeled MSCs a down regulation on day 2 and 6 after incubation could be observed in comparison to unlabeled cells. In Prussian blue staining single cells were detected which show a high iron-load up to 14 days after particle removal in comparison to other MSCs in the same culture.

Discussion: Iron-PLLA particles are well taken up by MSCs and do hardly alter the cell properties. The high ionic relaxivity of the optimized particles in combination with the very efficient uptake of the particles in the cells makes the investigated particles a promising candidate for sensitive cell labelling for MRI investigations.