# Nanoparticles optimized for efficient stem cell labeling and possessing optimal contrast properties for MRI and MPI

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## Introduction & Objective

Stem cell research in the field of regenerative medicine has increased significantly over the past few years because of the cells' healing capacities in neurodegenerative diseases such as Parkinson's or Alzheimer's disease. Monitoring and homing of stem cells *in vivo* is essential for improved understanding of their interactions within the organism and their therapeutic effects. In this work, we present negatively charged iron oxide nanoparticles, which are optimized for efficient stem cell labeling and possess optimal contrast properties for magnetic resonance imaging (MRI) as well as for magnetic particle imaging (MPI).

## Particles & Methods

N7B_CD particles are carboxydextran coated and narrowly size distributed iron oxide nanoparticles optimized for stem cell labeling. The particles are based on FeraSpin™, a contrast agent which is already used in various MRI and MPI studies. Relaxation times were measured at 0.94 T @ 39 ° C using a Bruker MiniSpec mq40 (Bruker BioSpin MRI GmbH; Germany), gel phantoms were imaged with a 1 T small animal imaging device (nanoScan®, Mediso GmbH, Germany) using a T2* weighted gradient Echo SNAP (GRE SNAP) sequence. The magnetic particle spectra (MPS) of the particles as well as of the labeled cells were recorded (20 mT/μ0, $f_0$ = 20 kHz) with a commercial MPS system (Pure Devices).

## Stem cell labeling & Assays

*In vitro* labeling of mesenchymal stem cells (*MSC´s*) was performed in DMEM medium by incubation of $1.5*10^5$ cells in a 6-well plate under standard cell culture conditions with the respective particle dispersion having an final iron concentration of 0.1 mg/mL. After incubation the cells were purified in order to remove non-incorporated particles. Subsequently, the iron content per cell was determined using an photometric phenanthroline assay. In order to assess potential cell toxicity, an MTT assay, which detects mitochondrial activity as a measure of cell viability, was performed.

## Results

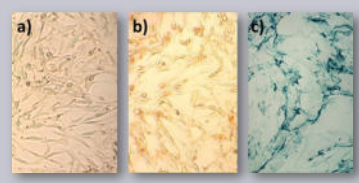
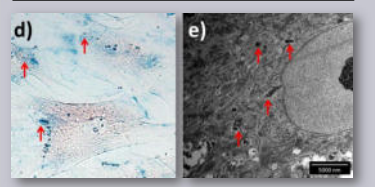
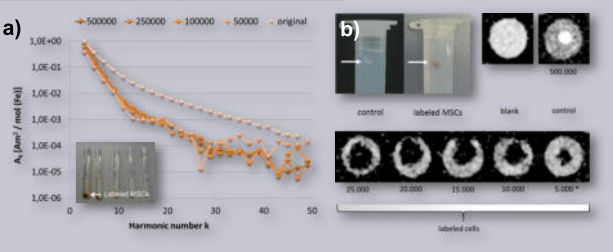



***Fig. 1:*** *Microscopic images of MSC´s a) before labeling b) after labeling with N7B_CD (0.1 mg/mL iron for 24 h) and c) after Prussian blue staining. Intracellular uptake confirmed by d) confocal and e) electron microscopy (iron containing vesicles are marked with the red arrows).*




Nanoparticles with a negative surface potential ζ of - 22 mV and a hydrodynamic mean diameter of 64 nm were used for *in vitro* labeling experiments. The particles are formulated according to physiological conditions and finally autoclaved. The contrast properties in MRI are found to be highly improved ($R_2/R_1$ = 22) compared to Resovist® ($R_2/R_1$ = 11). The MPS data show an increase up to a factor of three for the amplitude of the third harmonic with a less decay at higher harmonics. In order to demonstrate the superior contrast properties, MRI and MPS gel phantoms of labeled cells were prepared. MPS measurements (Fig. 2 a) clearly show that the amplitudes of the harmonics of N7B_CD labeled cells are reduced compared to particles measured in solution, explainable with changes in viscosity (water vs. cell compartments) and therefore changes in Néel and Brownian relaxation processes.[1] However, a cell phantom with 50000 cells was still able to be measured with sufficient amplitudes. T2*-weighted images of the MR phantom in Fig. 2b show the typical darkening originated by the iron oxide nanoparticles. A phantom with only 5000 labeled cells was able to be visualized and offers the possibility to monitor therapeutic effects *in vitro* as well as *in vivo*. The stem cell uptake of the N7B_CD particles is shown in *Fig. 1b* with the brownish color after incubation. Additional Prussian blue staining after removing the unbounded particles confirms the findings. The intracellular uptake of the particles was shown by confocal and electron microscopy (TEM) by the existence of iron containing vesicles (Fig. 1d and e). With 54 pg iron / cell N7B_CD shows an almost double as high iron uptake compared to Resovist. Because of the excellent iron uptake of the negatively charged particles the magnetically labeled cells can be easily attracted by an external magnetic field (*Fig. 4*). This enables the possibility of capture and transport the stem cells through the circulatory system to damaged tissue and organs improving homing and increasing the healing potential. In order to assess potential cytotoxic effects of the particles, an MTT test was performed. The results show no cytotoxic effects for the N7B_CD particles after 4 h and 24 h incubation (*Fig. 3c*).

***Fig. 2:*** ***a)*** *Odd harmonics of the MPS of N7B_CD labeled cells measured at an excitation field amplitude of 20 mT/μ0 and an excitation frequency of $f_0$ = 20 kHz in comparison with particles in solution (original).* ***All spectra were normalized to 1 mol Fe.*** ***b)*** *MR images of gel phantoms without cells (blank), with unlabeled cells (control) and with different numbers of labeled cells. One cell phantom is 10 mm in diameter. Relevant particle parameters are summarized in the table below.*




| | $d_h$ | PDI | ζ-Pot @ pH = 7 | $R_1$ | $R_2$ | $R_2/R_1$ | A3 | A11 | $A3_S/A3_R$ | $A11_S/A11_R$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Resovist® | 62 | 0.200 | -25.6 | 20.0 | 219.3 | 11.0 | $3.42x10^{-1}$ | $1.47x10^{-2}$ | 1.0 | 1.0 |
| N7B_CD | 64 | 0.214 | -22.0 | 13.4 | 304.5 | 22.8 | $9.42x10^{-1}$ | $4.13x10^{-2}$ | 2.8 | 2.8 |

*Hydrodynamic diameter $d_h$ in [nm], Relaxivity values R in [L/mmol*s], Amplitude A in [$Am^2$/mol Fe], Subscripted character: S = sample, R = Resovist®*

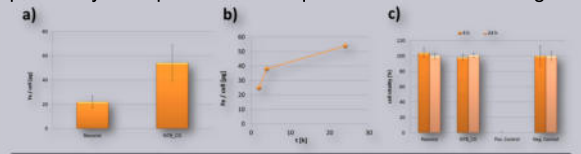



***Fig. 3:*** *a) iron uptake per cell of different particles after incubation for 24 h , b) iron uptake per cell over time of N7B_CD and c) MTT test of different particles. In all experiments a final iron concentration of 0.1 mg/mL iron was used.*

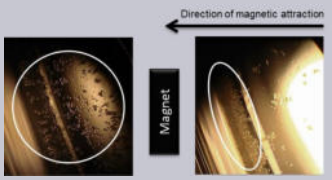



***Fig. 4:*** Interaction of magnetically labeled MSC´s with an external permanent magnet. Without a magnet the cells are randomly distributed (left figure). Applying the magnetic field leads to a movement into the direction of the magnet (right figure).

## Conclusion

In this study we report on highly effective and biocompatible iron oxide nanoparticles for *in vitro* stem cell labeling with optimized contrast properties for MRI and MPS. The particle characteristics allow a direct labeling without the use of any transfection agents or other additional treatments, which is favorable for the future development of clinical applications. The optimal iron uptake characteristics enable the use of external magnetic fields to influence the labeled cells, allowing not only their homing to a specific target but also capturing of the labeled stem cells *in vivo*.

## References & Acknowledgement

[1] F. Fidler, M. Steinke, A. Kraupner, C. Gruettner, K.H. Hiller, A. Briel, F. Westphal, H. Walles, and P.M. Jakob, "Stem Cell Vitality Assessment Using Magnetic Particle Spectroscopy." Magnetics, IEEE Transactions on, 51(2), 1-4, 2015.

**This work was supported by the European Commission's Seventh Framework Program – Health.2011.1.4-2 - Contract no. 279288 - IDEA**