

Supporting Information

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Manganese-Zinc Ferrites: Safe and Efficient Nanolabels for Cell Imaging and Tracking In Vivo

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Novel manganese-zinc ferrites (Mn-Zn ferrites) represent potentially interesting nanoparticles for MR imaging and cell tracking. The paper summarizes *in vitro* and *in vivo* nanoparticle examinations. This document provides supporting information.

Relaxivity

The key property for nanoparticles used as a cell label or a contrast agent is their relaxivity.

High r_2 relaxivity (Figure S11) corresponds to high magnetization of the nanoparticles. A strong temperature dependence of r_2 relaxivity was observed; nevertheless, Mn-Zn ferrite nanoparticles still possess, at body temperature, approximately two times higher relaxivity ($295 \text{ s}^{-1}/\text{mM}$) than commercially available iron oxide based particles.

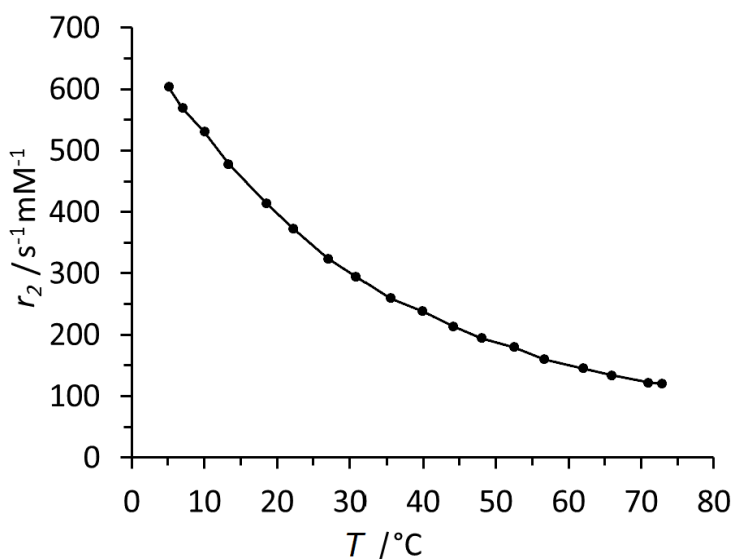


Fig. ES1: The transverse relaxivity r_2 of silica-coated $\text{Mn}_{0.61}\text{Zn}_{0.42}\text{Fe}_{1.97}\text{O}_4$ nanoparticles as a function of temperature T ($B_0 = 0.5 \text{ T}$).

In vitro cell tests

Apoptosis detection

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) revealed no apoptosis increase in both types of tested cells, even at the highest concentration, see Figure ES2. Cytometry charts are shown in Figure ES3.

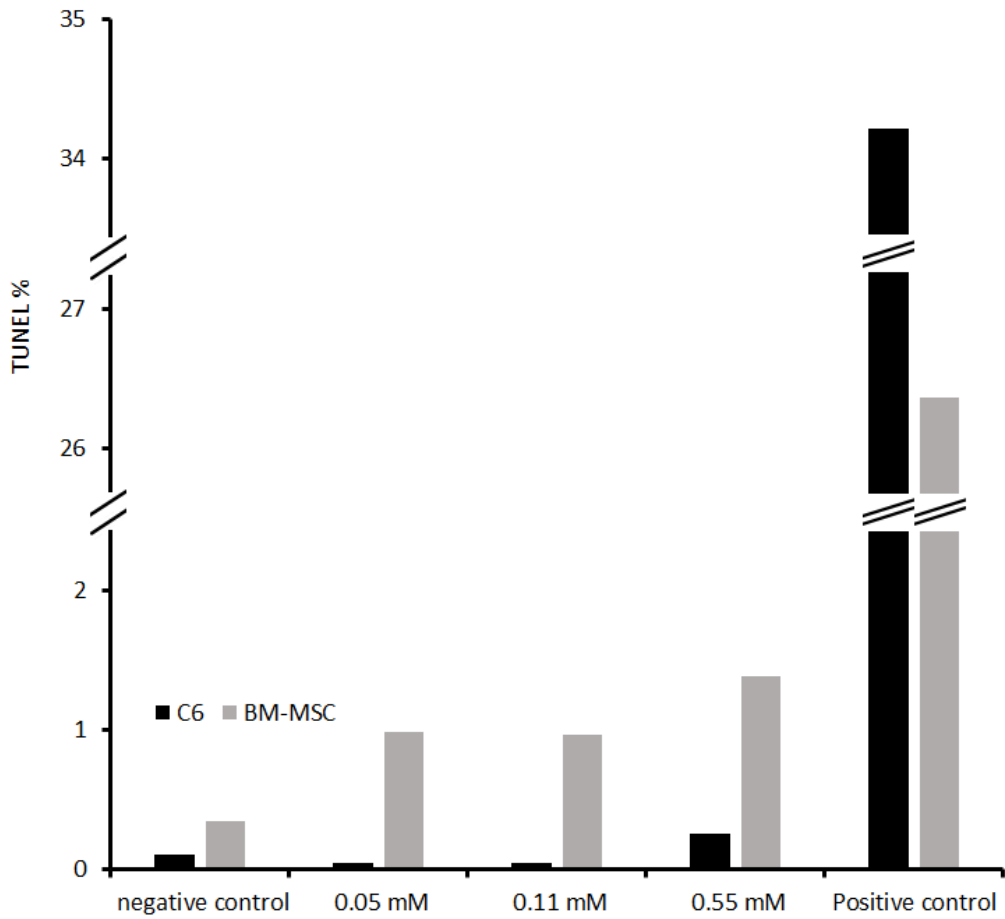


Fig. ES2: Apoptosis results for cells labeled with different concentrations of silica-coated MZF nanoparticles. Cells were incubated for 48 h and then labeled with FITC-dUTP; the proportion (in percents) of FITC-positive apoptotic events within nucleated singlet cells was determined. The negative control represents unlabeled stained cells, while the positive cells are apoptosis-induced cells included in the kit.

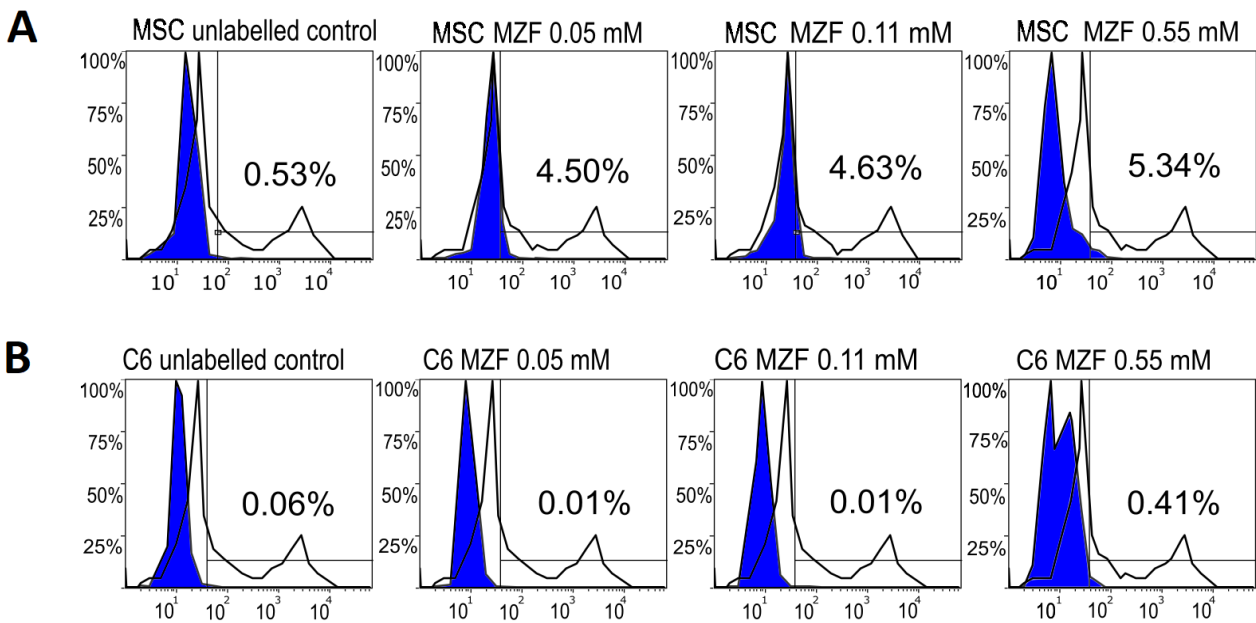


Fig. ES3: Flow cytometry after terminal deoxynucleotidyl transferase dUTP nick end labeling revealed no apoptosis increase in the negative control and labeled cell samples, either in MSCs (A) or in C6 cells (B). Apoptosis was detected in the positive control samples only. The unlabeled control and labeled samples (blue areas in the histograms) were overlaid with the positive control (empty area under the curve).

Reactive oxygen species production in labeled cells

All cells stained with CellROX® Deep Red reagent revealed a slight production of ROS, which was comparable with the untreated control. The production increase was not linear to the time of exposition to the nanoparticles. The proportion of dead cells was low and independent to ROS production, except for the positive control. However, in this positive sample, the dead cell count was not as high as expected, documenting a superior resistance of rMSC to ROS (Figure ES4).

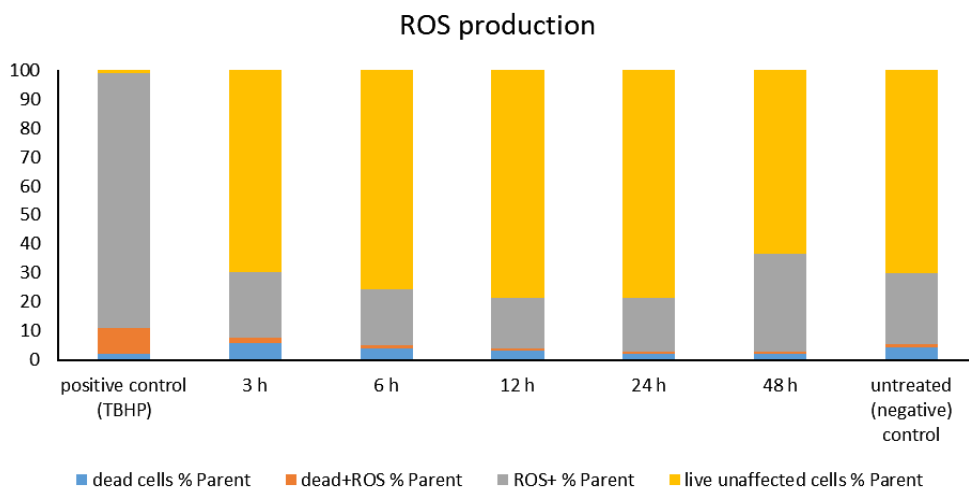


Fig. ES4: Percentage of unaffected rat mesenchymal stem cells (yellow), dead ones (blue), ROS positive (grey), and dead ROS positive ones after exposition to the nanoparticles for 3 to 48 hours.

In vitro MR relaxometry of cell suspensions and cellular metal content

Dependence of the R_2 relaxation rate of the labeled cells related to 1 million of cells per 1 mL on magnetic field strength is shown in Fig. ES5. Cells labeled at a 0.2 mM ($\text{Mn}_{0.61}\text{Zn}_{0.42}\text{Fe}_{1.97}\text{O}_4$) concentration in the culture medium showed a relaxation rate of $6.1 \text{ s}^{-1}/(10^6 \text{ cells/mL})$ at 4.7 T field strength.

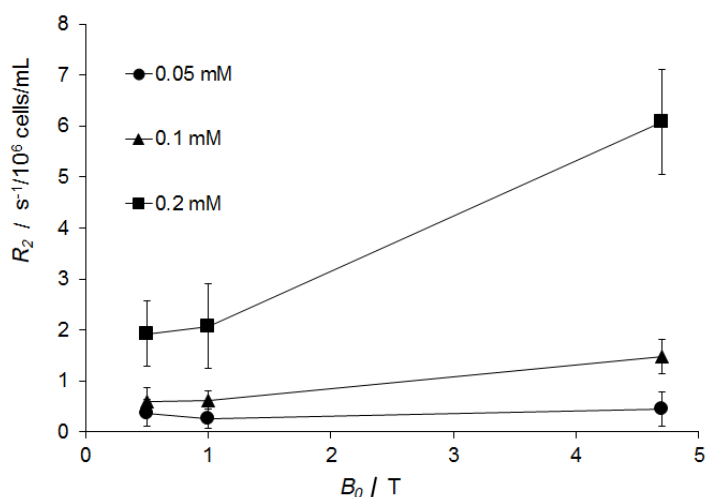


Fig. ES5: Dependence of the R_2 relaxation rate of cells labeled by silica-coated MZF nanoparticles under magnetic field strength B_0 at room temperature.

***In vivo* cell transplantation**

The cells were successfully transplanted into the muscle of a rat and the transplant was monitored by optical and MR imaging *in vivo*.

***In vivo* optical imaging**

The transplanted cells (both labeled and unlabeled) produced a bioluminescent signal after the intravenous application of luciferin, which confirmed that the grafts were viable.

The highest bioluminescent signal was visible on Day 1 (Figure SI6), then the signal gradually decreased down to 10 % within one week after cell transplantation, in both the labeled and unlabeled grafted cells. We presume that the vanishing of the bioluminescence in time corresponds to graft rejection, as no immunosuppression was used in the experiment. We may rule out nanoparticle toxicity, as similar decay in the signal was observed in both the labeled and unlabeled cells.

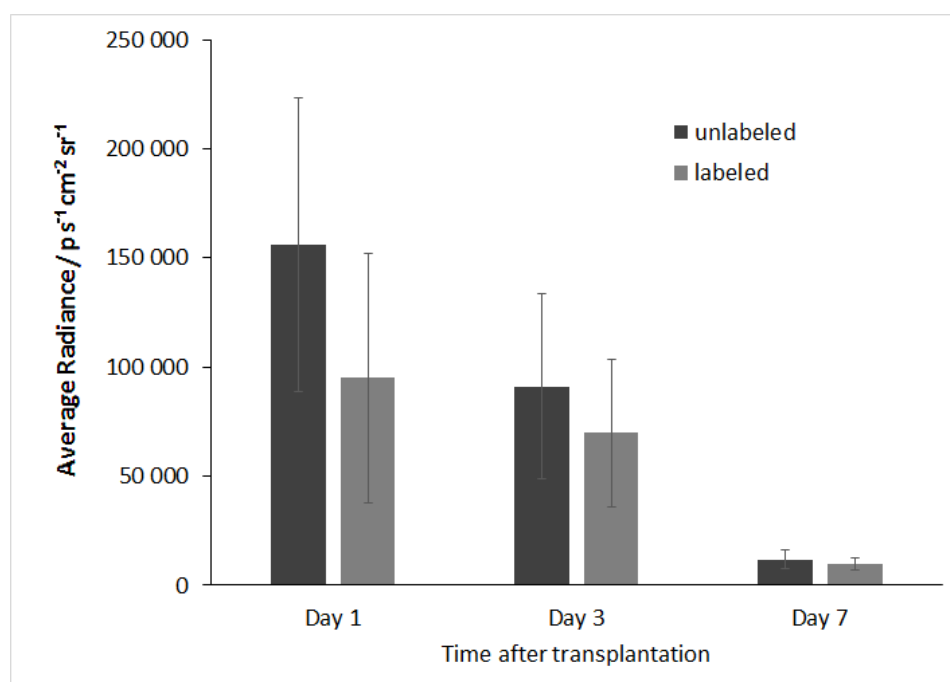


Fig. ES6: The decrease of the bioluminescent signal due to graft rejection. Unlabeled cells – black columns, cells labeled at 0.2 mM concentration – gray columns.

Although the bioluminescent signal intensity may reflect both cell the viability and signal quenching caused by the nanoparticles similar to *in vitro* observations, we also observed some variations of the bioluminescent signal of a rather stochastic nature during the experiment. We assume that this may reflect different absorption of the light in the tissue due to the different depth of the implantation site. Therefore, the signal intensity strongly depends on the actual position of the animal in the imager. An example of an animal measured at two slightly different positions (Figure SI7a-b) thus confirms that exact quantification of the surviving transplanted cells is not possible. The effect of light absorption in the tissue, combined with the different position of the animals during the examination period, caused a high dispersion of radiance in Figure ES6.

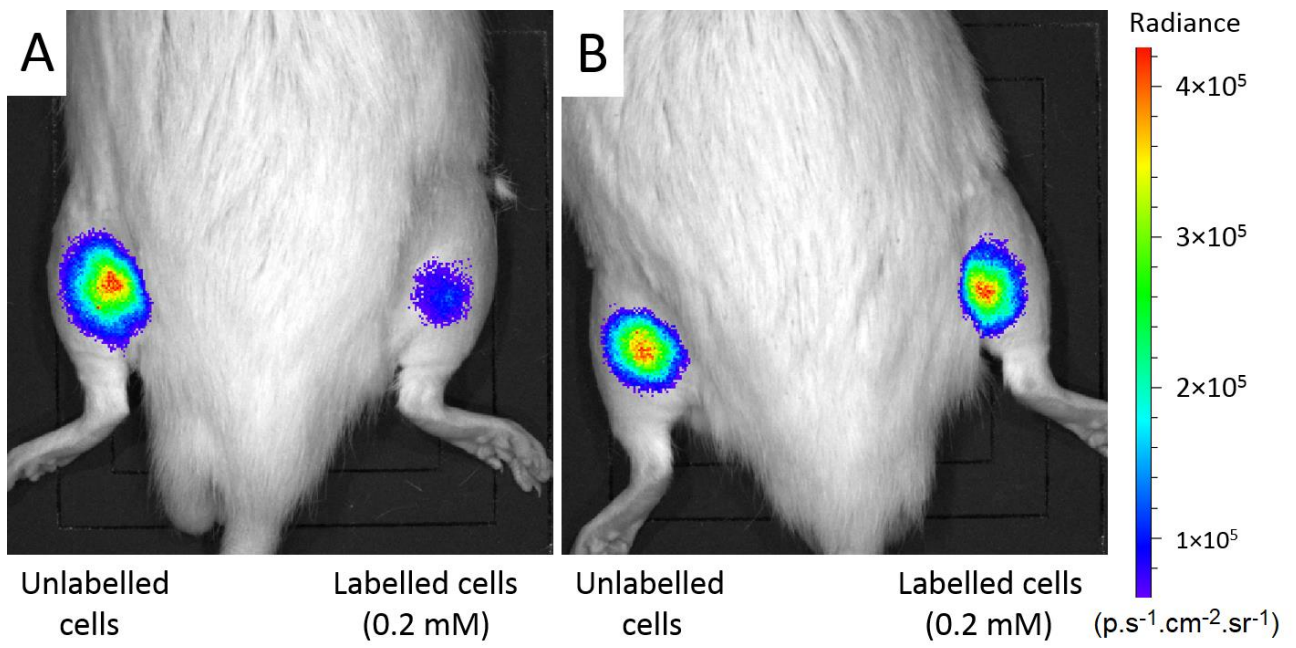


Fig. ES7: Bioluminescent signal from the engrafted cells in an animal with transplanted unlabelled cells (left leg) and cells labeled at 0.2 mM concentration (right leg) scanned before (a) and after (b) repositioning.