The effect of different magnetic nanoparticle coatings on the efficiency of stem cell labeling

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Introduction

Superparamagnetic iron oxide nanoparticles can be used for cell labeling and tracking, and as magnetic resonance contrast agents they are very important in the emerging fields of nanomedicine and nanoscience. They improve the detection and characterization of a local area by changing the signal intensity of target tissues when compared with the surrounding tissue. One of the most commonly used ferric oxide particles are maghemite (-Fe_2O_3) nanoparticles, due to their ability to be well tolerated by living organisms.

Materials and Methods

Magnetic nanoparticles

To optimize the uptake of magnetic nanoparticles into stem cells, various organic coatings have been developed in the Institute of Macromolecular Chemistry, AS CR.

Fig. 1. Chemical structure of Dextran (a), D-mannose (b), polydimethylacrylamide (c), poly-L-lysine (PLL) (d).

Cell culture and stem cell labeling

As a model for cell labeling we used rat (1st to 5th passage) and human (2nd to 4th passage) bone marrow mesenchymal stem cells (MSCs).

The cells were labeled with maghemite nanoparticles coated with poly-L-lysine (PLL), D-mannose-, or poly-dimethylacrylamide. As a control we used either the commercial contrast agent Endorem® or uncoated -Fe_2O_3. Both rat and human MSCs were incubated with a nanoparticle suspension at a concentration 15.4 μg Fe/ml. After 72 h, the ferric oxide particles were washed out by the replacing the culture medium.

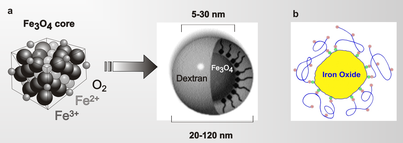
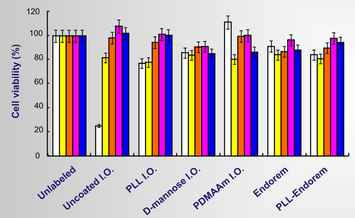


Fig. 2. Schematic illustration of iron-oxide nanoparticles. The contrast agent Endorem® consists of a superaramagnetic Fe_3O_4 core that is coated by a dextran shell (a). Interaction between PLL and a citrate-treated iron oxide nanoparticle (b).

Results

Cell viability



The viability of rat and human MSCs was assessed using a WST-1 colorimetric assay, based on the cleavage of the tetrazolium salt (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) to a highly water-soluble formazan dye by mitochondrial dehydrogenases in viable cells.

Fig. 3. Viability of unlabeled rat MSCs from the 1 st to 5 t h passages (left to right columns, respectively), labeled with uncoated iron oxide, PLL-, D-mannose-, and PDMAAm- coated iron oxide nanoparticles, Endorem® and PLL-modified Endorem®.

Staining intensity

Labeling efficiency was determined by manually counting the number of Prussian Blue-stained and unstained nanoparticle-labelled cells.

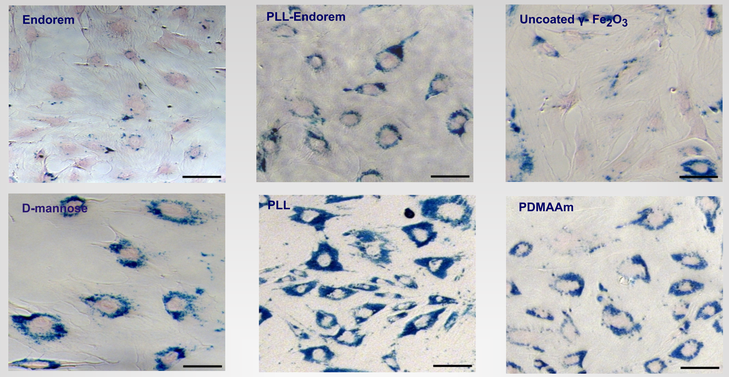
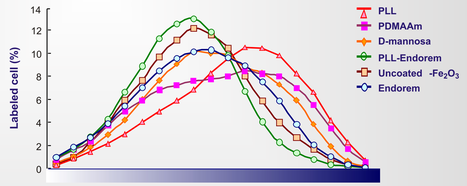


Fig. 4. Photomicrographs of Prussian Blue stained rMSCs labeled with Endorem® (a), PLL-modified Endorem® (b), uncoated -Fe_2O_3 (c) , D-mannose-coated -Fe_2O_3 (d), PLL-coated -Fe_2O_3 (e) and PDMAAm-coated -Fe_2O_3 (f) nanoparticles. Counter stained with nuclear fast red, scale bar 100 μm.

Distribution of the intensity of the Prussian Blue staining

Fig. 5. Distribution of the intensity of Prussian Blue staining (the x-axis shows gradient of Prussian Blue staining) in rMSCs. The percentage of intensely stained rMSCs was highest in cells labeled with PLL-coated -Fe_2O_3 nanoparticles, followed by PDMAAm-coated ones, D-mannose-coated ones, PLL-coated Endorem®, uncoated -Fe_2O_3 nanoparticles and Endorem®.



Labeling efficiency

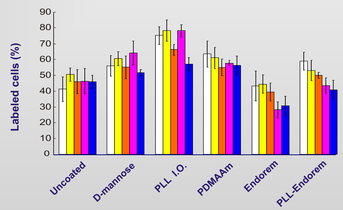
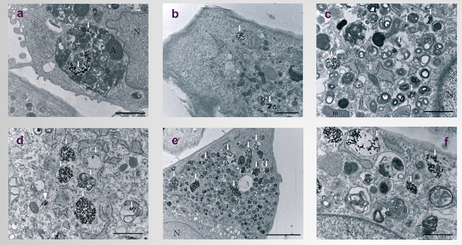


Fig. 6. Labeling efficiency of rat MSCs from the 1 st to 5 th passages (left to right columns, respectively) with uncoated iron oxide, D-mannose-, PLL-and PDMAAm-coated iron oxide nanoparticles, Endorem® and PLL-modified Endorem®.

Tab. 1.
Percentage of human and rat MSCs labeled with differently coated -Fe_2O_3 nanoparticles.

Nanoparticles	Labeled cells (%)	
	hMSCs	rMSCs
Uncoated -Fe_2O_3	74 6	44 5
Mannose-coated -Fe_2O_3	84 5	57 6
PLL-coated -Fe_2O_3	84 4	72 5
PDMAAm-coated -Fe_2O_3	77 6	59 6
Endorem	62 5	37 6
PLL-coated Endorem	77 5	49 9

The internalization of magnetic nanoparticles



The internalization of coated -Fe_2O_3 nanoparticles by rat and human MSCs was verified on 65-nm-ultrathin sections of cells embedded in epoxy resin using TEM. Large numbers of free magnetic nanoparticles were mostly seen inside numerous vacuoles.

Fig. 7. TEM micrographs of rMSCs labeled with (a) uncoated γ-Fe_2O_3, (b) Endorem®, (c) PLL-coated Endorem®, (d) D-mannose-, (e) PLL-and (f) PDMAAm-coated γ-Fe_2O_3 nanoparticles (arrows). Arrows and arrow head indicate nanoparticles inside the endosome/autophagosome and in the cytoplasma, respectively. A-autophagosome, m-mitochondrion, N-nucleus, n-nucleolus. Scale bar (d,f) 1 μm, (a,b,c) 2 μm, (e) 5 μm.

In vitro MR imaging of cell suspensions

To check the sensitivity of the magnetic resonance imaging (MRI) using a 4.7T Bruker spectrometer and to compare different types of -Fe_2O_3 nanoparticles to commercially available Endorem®, rMSCs were labeled with nanoparticles and suspended in gelatin.

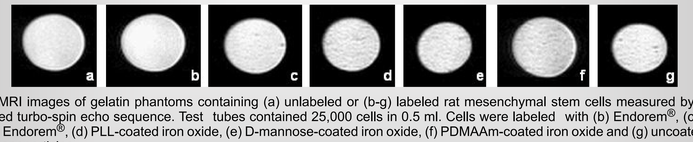


Fig.8. MRI images of gelatin phantoms containing (a) unlabeled or (b-g) labeled rat mesenchymal stem cells measured by a T2-weighted turbo-spin echo sequence. Test tubes contained 25,000 cells in 0.5 ml. Cells were labeled with (b) Endorem®, (c) PLL-coated Endorem®, (d) PLL-coated iron oxide, (e) D-mannose-coated iron oxide, (f) PDMAAm-coated iron oxide and (g) uncoated iron oxide nanoparticles.

Conclusions

- The mechanism of nanoparticle internalization into the cells differed depending on the type of coating
- Surface modification of iron oxide nanoparticles with D-mannose, PLL or PDMAAm had a strong impact both on their uptake by the cells and on the cell viability
- A higher percentage of labeled cells was found in hMSCs than in rMSCs
- The best efficiency of labeling (70-80 %) was achieved with PLL-coated -Fe_2O_3 nanoparticles
- Lower passages were labeled more than higher ones
- Newly synthesized particles represent a significant improvement in the cell detection using MRI
- Coated nanoparticles are a promising tool for the real-time tracking of grafted cells as well as the

Acknowledgements

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