Magnetic resonance imaging of adipose-derived adult stem cells labelled with superparamagnetic iron oxide nanoparticles

A.M. Capuzzo[1], I. Scambi[2], R. Mariotti[2], A. Lacavalla[3], T. Knickrehm[4], K. Lüdtke-Buzug[4], P. Marzola[3]

1Department of Diagnostics and Public Health University of Verona, Verona, Italy
2Department Neurosciences, Biomedicine and Movement Sciences University of Verona, Verona, Italy
3Department of Engineering for Innovation Medicine, University of Verona, Verona, Italy
4Institute of Medical Engineering, University of Lübeck, Lübeck, Germany

E-mail: arnaudmartino.capuzzo@univr.it

Introduction

Background. The application of mesenchymal stem cells (MSCs) represents a new promising approach for treating neurodegenerative diseases. Recently, considerable attention has been paid to adipose-derived adult MSC (ADAS), thanks to the easy availability of adipose tissue and to the possibility of autologous cells transplantation[1]. Any possible application of therapies based on ADAS in the clinics cannot occur without elucidation of their homing[2]. Superparamagnetic iron-oxide nanoparticles (NPs) can be used to label and track cells in vivo via Magnetic Resonance Imaging (MRI)[3]. The accessibility, non-invasiveness, and radiation-free features of MRI make this imaging tool appropriate for translation to the clinics[4]

Aim. Validate an ADAS labelling system that allows us to trace them as homing in MRI and to trace them for their path in relation to the progress of the neurodegenerative disease.

Materials and Methods

Isolation of adipose murine cells from C57BL/6. The designated adipose tissue depots were taken from the posterior subcutaneous location composed of dorsolumbar and inguinal depot. Extracellular matrix was digested for 45 min at 37° C in HBSS containing 1 mg/ml of collagenase type I and 2% (w/v) BSA for 45 min. Depots were centrifuged 10 min at 1200 × g, room temperature, to obtain a high-density pellet that constitutes the stromal vascular fraction (SVF). SVF were resuspended in 160 mM NH4 Cl and incubated at room temperature for 10 min to lyse contaminating red blood cells. SVF were centrifuged 10 min at 1200 × g, room temperature and filter (with a 70-µm nylon mesh cell strainer) to remove cell debris. The final step was to put in culture SVF in 25-cm2 flasks in complete DMEM medium at 37° C in a 5% CO2 for 3 days.

Cultivation of adipose derived adult stem murine cells (ADAS). ADAS were cultured with complete DMEM containing 10% FBS and 1% P/S mix 1:1 and incubated at 37° C in a 5% CO2 atmosphere. When ADAS were at 70% to 80% confluence, we wash with DPBS (without Ca or Mg) and trypsinized (0.05% trypsin at 37° C for 5 min). Then we harvested and washed with medium to remove trypsin and cultured in larger flasks with the cell number needed for the experiments.

Characterization of Nanoparticles for Biophysics detection. To label ADAS we used two NPs: L-NP, synthesized by the Lübeck group and Resovist® ®, commercially available, used as reference. Both NPs were characterized from a biophysical point of view with: Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Nano Sight and MRI. L-NPs sizes determined by DLS (~53.02 nm), TEM (~100 nm) and Nano Sight (~93.9 nm) were distinct from Resovist® ® (DLS ~157.67 nm, TEM ~70 nm and Nano Sight ~56.7 nm) and were somewhat dependent on the solvent. MRI transversal relaxivity of L-NPs 277.8 mM-1s-1 was higher than Resovist® ® 156.4 (mM-1s-1).

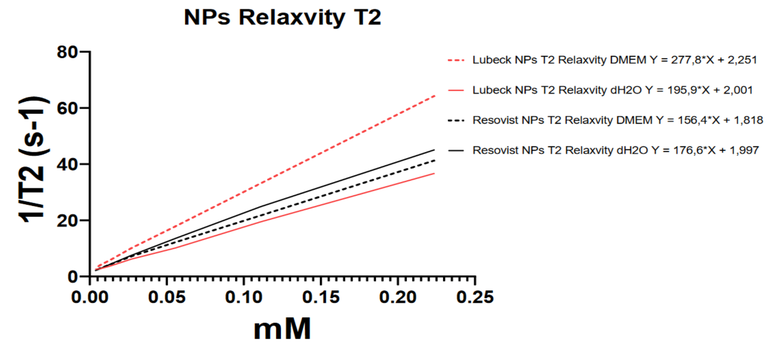
Labelling and Vitality with Lubeck and Resovist® NPs. MTT test was performed to evaluate metabolic activity of labelled ADAS in culture. ADAS cells were seeded in 96 wells with 100 µl DMEM 10% FBS 1% P/S 1:1 mix. Three time points were established (24h, 48, 72h) to investigate the labelling with NPs Lubeck (stock solution, 22.7 mg Fe/ml) Resovist® (stock solution, 28 mg Fe/ml). Labelling was set at (µg Fe/ml) 200; 100; 50; 25; 12.5 and 6.2. Results are presented as relative metabolic activity in comparison with cells without NPs, considered to be 100% vital.

MRI Phantoms on Lubeck and Resovist® NPs. To test the efficiency of NPs as an MRI contrast agent in a biologically significant context in order to identify the minimum number of labelled cells**,** ADAS cells were seeded in T-25 with 5 ml DMEM 10% FBS 1% P/S 1:1 with different NPs concentration (µg Fe/ml 200, 50 and 6.2). Different cells concentration were investigated (10, 50, 100, 10^3, 5x 10^3, 10^4, 5x 10^4, 10^5) to detect the lowest iron signal. Acquisition of FLASH sequence images of ADAS-NPs were made a 24, 48 and 72 h. MR images were acquired with a 7T Bruker Biospec scanner.

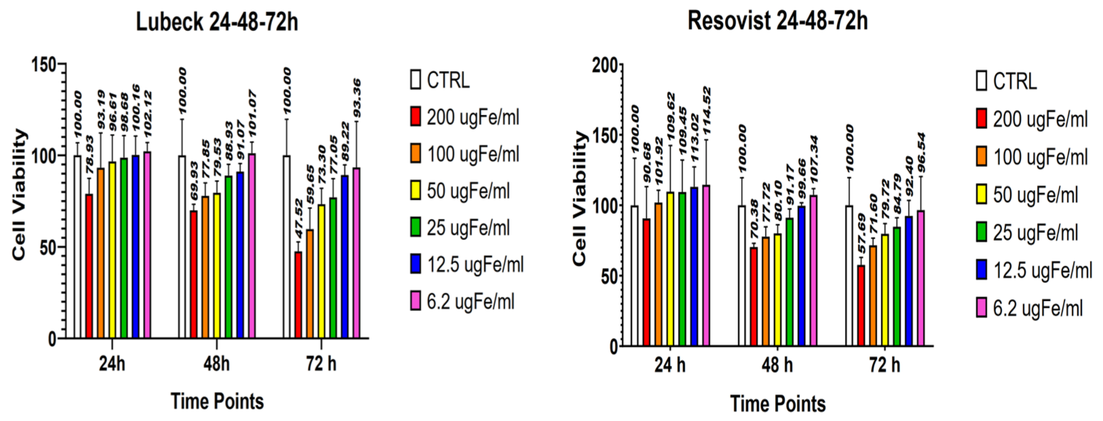
Prussian Blue on Lubeck and Resovist® NPs. To verify the internalization of NPs, cells were stained with Prussian Blue and counterstained with nuclear fast red, 2x 10^4 ADAS cells were seeded onto 13mm coverslips, placed in 24-well plates, and NPs (µg Fe/ml 200, 50 and 6.2) were added to culture media after 24h. Cells were incubated with NPs for 24 and 48h. Cells were washed with PBS and fixed with 4% PFA. To verify the internalization of NPs, cells were stained with Prussian Blue and counterstained with nuclear fast red and observed using Olympus BXS1 light microscope.

Results

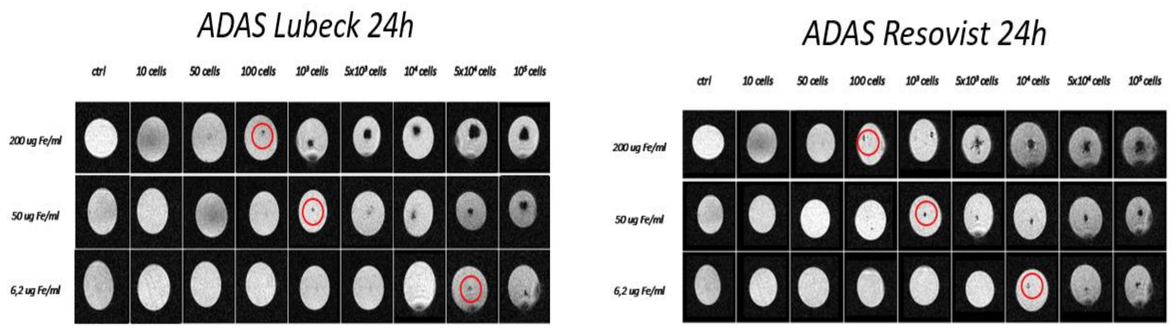
Characterization of Nanoparticles for Biophysics detection. The ability of NPs to shorten the transversal relaxation time of nearby water protons during MRI was obtained by a serial dilution from a solution containing µg Fe/ml 200 to 0. The results showed the contrast agents ability, with a Lubeck transverse relaxivity r2 of 277.8 + 2.251 1/T2 (s-1) in DMEM and Resovist® transverse relaxivity r2 of 156.4 + 1.818 1/T2 (s-1) in DMEM. Relaxivity were determined in medium DMEM and distilled water solution, 37 ° C at Biospec Tomograph (Bruker Medizintechnik GmbH, Karlsruhe, Germany) equipped with a 7-tesla (T) horizontal magnet.



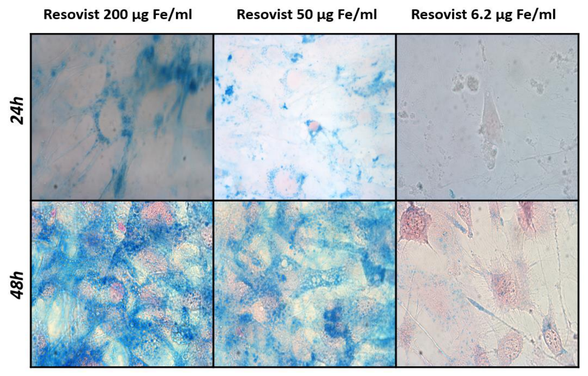
Labelling and Vitality with Lubeck and Resovist® NPs. Tests of viability of the labelled cells, by MTT, showed that L-NPs up to 100 µg Fe/ml can be used for cell labelling.



MRI Phantoms on Lubeck and Resovist® NPs. To investigate the detectability of ADAS-NPs, MR images of agarose gel phantoms containing increasing number of labelled cells were acquired. Preliminary results showed that as few as 100 labelled cells can be tracked using L-NPs.



Prussian Blue on Lubeck and Resovist® NPs. Prussian Blue staining was performed and stained ADAS cells were observed using a light microscope Olympus BXS1. Labelled cells were stained after 24 and 48h of incubation with 200, 50 and 6.2 µg Fe/ml of each NPs in order to evaluate the cellular uptake. Prussian Blue on Lubeck NPs is in course.



Discussion

An ADAS stem cell labelling system has been validated with the use of superparamagnetic nanoparticles in order to be able to visualize homing through magnetic resonance, allowing us to continue further studies investigation.

References

1.Turano E. et al. Int J Mol Sci. 2023;24(3):2917.
2.Capuzzo et al. XXVII SCHOOL OF PURE AND APPLIED BIOPHYSICS Extracellular vesicles: from biophysical to translational challenges. 2023.
3.Busato A. et al. Curr Protoc Cell Biol. 2017;75(1):3.44.1-3.44.15.
4.Bontempi P. et al. Magn Reson Med. 2018;79(1):459-469.

Take Home Message

A labelling system with the use of nanoparticles combined with stem cells can become a non-invasive theragnostic method thanks to the use of magnetic resonance imaging. The accessibility, non-invasiveness, and radiation-free features of MRI make this imaging tool appropriate for translation to the clinics.