International Journal on Magnetic Particle Imaging
Vol 6, No 2, Suppl 1, Article ID 2009001, 3 Pages

Infinite Science Publishing

Proceedings Article

Encapsulation of new MPI tracer nanoparticles in the human red blood cells

A. Antonelli[1,*] · P. Szwargulski[2] · E. S. Scarpa[1] · C. Grüttner[3] · L. Guidi[1] · G. Ambrosi[1] · T. Knopp[2] · M. Magnani[1]

[1]Department of Biomolecular Sciences, University of Urbino Carlo Bo, Via Saffi 2, 61029 Urbino (PU), Italy
[2]Institute for Biomedical Imaging, Hamburg University of Technology, Am Schwarzenberg-Campus 3, 21073, Hamburg, Germany
[3]micromod Partikeltechnologie GmbH, Friedrich-Barnewitz-Str. 4, D-1811, Rostock, Germany
[*]Corresponding author, email: antonella.antonelli@uniurb.it

Abstract

Although Magnetic Particle Imaging (MPI) is not yet in clinical use, it is highly promising for several medical applications, and especially for applications in diagnostic vascular in vivo imaging and imaging-guided vascular interventions. Furthermore, in the last years, different superparamagnetic iron oxide (SPIO) based contrast agents have been developed and approved for niche clinical applications in Magnetic Resonance Imaging (MRI) as alternative to Gadolinium-based contrast agents (GBCAs) due to the risk for patients suffering from kidney dysfunction or nephrogenic systemic fibrosis (NSF). Recently, the potential of RBCs loaded with different SPIO nanoparticles as blood-pool tracer agents with longer blood retention time for MRI and MPI has been investigated. Here, we report the first in vitro results with the highly efficient dextran-based MPI tracer particles perimag® and synomag®-D to study their eligibility to be encapsulated into human RBCs and the potential of these new SPIO-RBC constructs as tracer material for MPI.

I Introduction

Several commercial iron oxide dextran nanoparticles with sizes in the range of 50-160 nm have been widely exploited for liver, spleen and lymph node imaging because they are recognized by the reticuloendothelial system (RES) and subjected to macrophage phagocytosis. Various biodistribution studies report a preferential accumulation in liver and spleen, moreover size, shape, and surface characteristics of iron oxide nanoparticles determine their biological distribution [1]. On the other hand, the rapid accumulation in the RES, abundantly present in the liver and spleen, limits the SPIO survival in blood circulation thus hampering their use for the vascular system diagnosis. Encapsulation of SPIO nanoparticles into red blood cells (RBCs) can increase the blood circulation time of nanoparticles [2]. The RBCs, thanks to their properties to be reversibly opened under hypotonic conditions without losing their natural features and functionalities, represent extraordinary intravascular carriers for drugs, biologics and other therapeutic agents. In the last years we attempted to identify between the commercial and the preclinical available SPIO nanoparticles those that can be efficiently loaded into RBCs. The use of RBCs as diagnostic tool is related to urgent identification of nanomaterial potentially suitable for producing SPIO-loaded RBCs with long half-life in blood circulation. The identification of this opens up a variety of MPI medical applications that need intravascular magnetic tracers to monitor cardiovascular interventions for a cardiac vitality evaluation, tissue perfusion, detection of bleeding sources and brain diseases such as stroke. Here, we re-

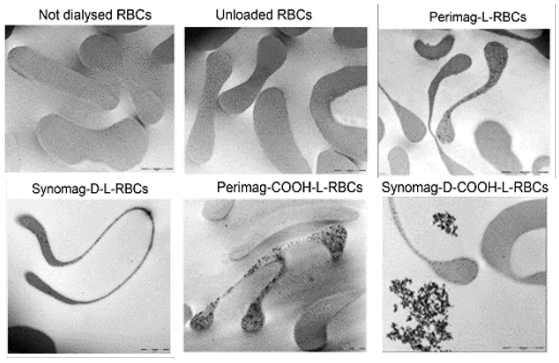

Figure 1: TEM images of micromod particles-loaded RBCs obtained dialyzing 1 ml of RBC70% in the presence of 11.2 mg Fe.

Table 1: T1 and T2 NMR measurements of RBCs loaded with perimag®, synomag®-D, perimag®-COOH, synomag®-D-COOH obtained dialyzing 1ml of RBC70% in the presence of 11.2 mg Fe. The Fe concentrations ([c]) encapsulated into all loaded RBCs were calculated with the following formula: $(1/T1c\text{-}1/T1_0)/r1$ or $(1/T2c\text{-}1/T2_0)/r2$.

Sample	T1 (ms)	T2 (ms)	mM (from r1)	MCV (fl)	MCH (pg)	MCHC (g/dl)	cell recovery (%)
ND	2476 ± 38	61.7±6.2	/	88	29	33.2	/
UL	2265 ± 95	62.4±7.4	/	70	20.6	29.6	70
Perimag®-L-RBCs	1478 ± 64	16.2±0.7	0.58±0.10	60	12.7	21.1	60
Synomag®-D-L-RBCs	770 ± 8	5.4±0.6	0.90±0.12	65	12.3	20.8	63
Perimag®-COOH-L-RBCs	262.5 ± 42	14.31±0.9	6.54±1.10	68	18.6	27.4	51
Synomag®-D-COOH-L-RBCs	258.4 ± 15	21.97±1.1	3.88±0.20	68	17.1	25.1	57

port the first in vitro results obtained with the dextran-based MPI tracer particles perimag® and synomag®-D to study their eligibility for entrapment into human RBCs. In fact, these nanomaterials although biocompatible and with excellent properties in MRI and MPI, are rapidly taken up by the liver and spleen (for plain synomag®-D particles t1/2 < 4 minutes) when administred in vivo. Though other coating strategies, such as PEG coating, lead to synomag®-D nanoparticles survival in the vascular system for less than 2 hours [3-4].

II Material and methods

The erythrocytes loading procedure was performed with plain (non-modified) dextran particles: perimag® (product code; 102-00-132, 130 nm diameter) and synomag®-D (product code; 104-00-501, 50 nm diameter) obtained from micromod Partikeltechnologie GmbH. Corresponding COOH surface-functionalized perimag® (micromod product code; 102-02-132, 130 nm diameter) and synomag®-D particles (micromod product code; 104-02-501, 50 nm diameter) were tested to study the influence of surface charge on the encapsulation efficiency. Essentially, 1 ml of RBCs at 70% hematocrit were dialyzed in presence of magnetic nanoparticles using 11.2 mg Fe of all nanoparticle suspensions, following the same procedure previously reported [2]. Not dialysed RBCs (ND) were used as control as well as the unloaded erythrocytes (UL) that were prepared under the same conditions but dialyzed in absence of magnetic nanoparticles. Mean corpuscular volume (MCV), mean hemoglobin concentration (MCH), and mean corpuscular hemoglobin concentration (MCHC) were measured with an automated hemocytometer to evaluate the cell integrity. Moreover, control cells and SPIO-loaded RBCs were analyzed by Transmission Electron Microscopy (TEM). The amount of SPIOs incorporated in RBCs was assessed by T1 and T2 NMR relaxation measurements using a dose-response curve generated by adding known amounts of perimag® and synomag®-D contrast agents to human RBC samples at 44% of hematocrit. The measurements were performed at a magnetic field strength of 9.4 Tesla at 37°C using an Avance-400 NMR Bruker spectrometer [2].

III Results and discussion

The longitudinal (r1) and transversal (r2) relaxivities resulted in 0.8748 and 253.56 s^{-1} mM^{-1} for synomag®-D-plain, and 0.4972 and 219.95 s^{-1} mM^{-1} for perimag®-plain. All SPIO-loaded RBC samples showed a decrease of T1 and T2 values compared to control cells (ND; UL-RBCs), and this is more evident for synomag®-D-plain loaded RBCs sample (T1; 770 ms versus 2476 ms of ND and 2265 ms of UL-RBCs, Table 1) corresponding to 0.9 mM Fe concentration. However, the data obtained with these nanoparticles are different from those previously reported for Resovist® contrast agent that leads to a higher decrease of T1 and T2 (T1 ≤ 100 ms, T2 < 5ms) corresponding to an iron concentration approximately 4mM . When the same iron amount is used to perform the loading experiment (that is 11.2 mg Fe/ml RBC 70% hematocrit), the final iron encapsulation results in 0.9 mM if Synomag-D-plain is used and 4 mM if Resovist is used. Synomag®-D and perimag® loaded RBCs were slightly smaller on average than the untreated cells (MCV 61-73 fl versus 85 fl of ND RBCs) but similar to unloaded cells (70 fl). They have less hemoglobin per cell (MCH 13-17 pg versus 21 and 28 pg of unloaded and untreated cells) as well as a lower mean cellular hemoglobin concentration (MCHC 17-25 g dl-1 versus 31-33 g dl-1 of control cells). Synomag®-D and perimag® loaded RBCs as well as UL-RBCs appear to be similar in terms of cell recovery (60-70%). However, Fe amount seems to be higher in synomag-D®-loaded RBCs (0.49mM versus 0.23 mM in perimag® plain-loaded RBCs). Final Fe concentrations were around 1 mM for synomag®-D-loaded RBCs and 0.6mM for perimag®-loaded RBC samples. Figure 1 shows representative TEM images of synomag®-D- and

perimag®-loaded RBCs. prepared using the same iron amount. These images reveal different morphological features and distributions of iron oxide particles between synomag®-D- and perimag®-loaded RBCs.

Moreover, many iron oxide nanoparticles are also present in the extracellular space and this seems more evident in the synomag®-D-loaded RBC sample. COOH functionalized synomag®-D- and perimag® nanoparticles were also considered in order to evaluate if a more negative surface charge can affect the efficiency of SPIO encapsulation in the RBCs. Clearly, the loading procedure of synomag®-D- and perimag®-COOH particles resulted in samples with higher iron concentrations compared to plain particles-loaded RBCs (Table 1). Nevertheless, TEM analyses of synomag®-D-COOH loaded-RBCs showed the presence of iron oxides, often in big aggregates, not only into cells but also in the membrane and in the extracellular space (similarly as found in the synomag®-D-plain loaded RBCs sample, Figure 1). The COOH-surface of dextran coated perimag® and synomag®-D particles seems to improve the efficiency of encapsulation into human RBCs (6.5 mM Fe and 3.88 mM Fe for perimag®-COOH- and synomag®-D-COOH-loaded RBCs respectively, compared to 0.58 mM and about 1 mM for perimag® and synomag®-D-plain-loaded RBCs, Table 1). Particularly, representative TEM image reveals that perimag®-COOH nanoparticles are efficiently encapsulated in the RBCs (Figure 1). On the contrary, it appears that the superparamagnetic Fe content evaluated by NMR in the synomag®-D-COOH-RBC sample, mainly derives from the presence of extracellular iron oxides and not only from iron encapsulated inside the cells as shown in Figure 1.

IV Conclusions

Synomag®-D and perimag® bulk nanoparticles provide a two- to four-fold increase in MPI signal intensity compared to Resovist® as reported in ref. 3. Here, we proposed an approach to prolong their in vivo survival evaluating their encapsulation in human RBCs to produce new long-circulating biomimetic SPIO-carriers for MPI. Perimag®-COOH particles, that are nearly completely located inside the cell cytoplasm, perform better than all tested iron oxides. Synomag®-D-COOH particles are mainly present in the extracellular space. As the perimag® surface change led to an improvement of nanoparticle entrapment in the RBCs, we hypothesize that the optimization of synthesis protocols and surface changes of particles could be advantageous to produce new SPIO-RBC carriers. This is very important in the future perspectives of in vivo administration, since non-viable SPIO-RBCs are rapidly eliminated by the spleen and liver. Perimag®-COOH-loaded RBCs could be evaluated as new MPI tracer for future in vivo circulation studies.

Author's Statement

Research funding: EuroNanoMed III, MAGneTISe project. Authors state no conflict of interest.

References

[1] Qiyi Feng, Uptake, distribution, clearance, and toxicity of iron oxide nanoparticles with different sizes and coatings, Scientific Reports, 8(1), 2082, 2018.

[2] Antonelli, A et al. Characterization of ferucarbotran-loaded RBCs as long circulating magnetic contrast agents Nanomedicine (Lond) 11 (21): pp. 2781-2795, 2016

[3] C. Grüttner, synomag®Nanoflower Particles: A new Tracer for MPI, Physical Characterization and Initial in vitro Toxicity Studies, Book of Abstracts, 8th IWMPI, 2018, pp. 17-18, T. Knopp, T.M. Buzug Eds.

[4] Jeff M. Gaudet In vivo biodistribution and clearance of magnetic nanoflowers quantified with Magnetic Particle Imaging, 14th annual meeting of ESMI, March 19-22, 2019, Glasgow, UK.

