Biodistribution studies of polymer coated superparamagnetic iron oxide nanoparticles using Magnetic Resonance Imaging

Lamiaa M.A.Ali[1], Pasquina Marzola[2*], Elena Nicolato[2], Silvia Fiorini[2], Marcelo de las Heras Guillamón[3*], Rafael Piñol[1], Lierni Gabilondo[1], Angel Millan[1], Fernando Palacio[1*]

[1] Instituto de Ciencia de Materiales de Aragón, CSIC – Zaragoza University, Zaragoza, Spain.
[2] Department of Computer Sciences and Neurological Sciences, Neuropsychological, Morphological and Movement, Section of Anatomy and Histology, Verona University, Verona, Italy.
[3] Department of Animal Pathology, Unit of Histology and Anatomical Pathology, Zaragoza University, Zaragoza, Spain.

Miss_limo@yahoo.com, pasquina.marzola@univr.it, lasheras@unizar.es, palacio@unizar.es

Abstract

Magnetic resonance imaging (MRI) is considered as one of the most promising noninvasive diagnostic tools in medical science. Since it provides three-dimensional anatomical images with high spatial resolution in the sub-millimeter range [1]. Superparamagnetic iron oxide nanoparticles (SPIONs) are inorganic nanomaterials comprising a class of MRI contrast agents, as they enhance the image contrast and thus improve the sensitivity and specificity of MRI in mapping information from tissues. Thus far, several SPIONs preparations have already been used for clinical practice, especially for liver MRI, such as Ferumoxides (i.e. Endorem® in Europe, Feridex® in the USA and Japan) coated with dextran [2], and Ferucarbutran (i.e. Resovist® in Europe and Japan) coated with carboxydextran [3]. The magnetic nanoparticles studied here are part of a synthetic platform for SPIONs based on the use of polymers. They are made from biocompatible components, and are stable as a colloidal aqueous fluid in phosphate buffered saline (PBS) at pH = 7.4 and human body temperature [4]. This nanoplatform has an outstanding capacity for multifunctionalisation that, apart from magnetism has already been proved for: luminescence (fluorescein, rhodamine, and lanthanides), radiochemical tracing (In111), antibodies, molecular thermometry [5], and anticancer drugs (cis-Pt). They consist of: 1) hydrophobic polymer [poly(4-vinyl pyridine) (P4VP)] core encapsulating maghemite (γ-Fe_2O_3) nanoparticles, which is densely coated with 2) hydrophilic polymer chains [polyethylene glycol (PEG)] that may contain at the outer end various functionalities as mentioned above. In the sample used in this work, a part of the PEG chains are functionalized with –COOH groups to provide sites for the anchoring of antibodies or peptides to nanoparticle surface so they can be directed to specific targets. The iron oxide nanoparticles in the P4VP core have a diameter of 13 nm and they are embedded within the P4VP matrix, thus preventing agglomeration. The core and the polymeric coatings have a total hydrodynamic diameter of 163 nm.

Since the rationale of the synthesis of these ferrofluids is to improve current commercial contrast agents, *in vitro* relaxation measurements for ferrofluids as a function of core diameter have been carried out and compared to Endorem® [6]. In addition, the blood compatibility of the ferrofluids have been verified [7]

Longitudinal and transversal relaxation rates (1/T1 and 1/T2) were plotted as a function of iron concentration and r1 and r2 relaxivities were obtained by the slope of the fitting straight line. The performance of the ferrofluid as a contrast agent in cerebral perfusion experiments compared to Endorem® has been investigated. The mapping of the cerebral blood flow (CBF) and cerebral blood volume (CBV) were carried out following the bolus tracking method (first passage) [8]. Results clarify the effect of both Endorem® and ferrofluids on the signal intensity *in vivo*, however, Endorem® decreases the signal intensity more than ferrofluids. To evaluate the performance of the ferrofluid as a contrast agent compared to Endorem® in steady-state condition, T2*-weighted images in brain were acquired before (pre) and during 2h after (post) injection of ferrofluids and Endorem®. Results show that few minutes after injection of ferrofluids and Endorem®, a decrease in the signal intensity was observed for both contrast agents (Fig.1B, 1F), in contrast, at two hours after injection, a hypointense brain was observed for mice injected with Endorem® (Fig.1C), however, a cleared brain was observed for mice injected with ferrofluids that emphasizes the clearance of ferrofluids from the circulation (Fig.1G).

To evaluate the biodistribution of ferrofluids and Endorem® in the mouse body, T2 and T2*-weighted images were performed. T2 and T2*-weighted images were acquired before (pre), 5min-2h, 24h, 7days, 15days, 30days, and 60days after (Post) injection. Results show the accumulation of ferrofluids and Endorem® in the liver few minutes after injection, Endorem® is completely cleared from the liver 30 days after injection, however, ferrofluid is cleared from the liver 60 days after injection. No accumulation of ferrofluids and Endorem® was detected in kidney using MRI methods. The presence of ferrofluids and Endorem® in tissues (liver, kidney, spleen, lungs, and heart) at different time points (2 hours, 24 hours, 7 days, 15 days, 30 days and 60 days) was determined by Prussian blue assay. Furthermore, histological studies were carried out; results show that no significant histopathological changes were

detected Fig. 2. We can conclude that these ferrofluids act as long-term contrast agents without generating any notable histological lesions in mice organs over a period of 60 days.

References

[1] C.M. Lee, H.J. Jeong, E.M. Kim, D.W.Kim,S.T. Lim,H.T.Kim, I.K.Park, Y.Y.Jeong,J.W.Kim, M.H.Sohn, Magn Reson Med, **62**(2009)1440.
[2] R. Weissleder, D.D.Stark, B.L.Engelstad, B.R.Bacon, C.CCompton, D.L.White, P.Jacobs, J.Lewis, American Journal of Roentgenology, **152**(1989)167.
[3] P. Reimer, E.J. Rummeny, H.E. Daldrup, T. Balzer, B. Tombach, T. Berns, P.E. Peters, Radiology, **195** (1995)489.
[4] A. Millán A, F. Palacio, A. Falqui, E. Snoeck, V. Serin, A. Bhattacharjee, V. Ksenofontov, P. Gütlich, I. Gilbert, Acta Materialia **55**(2007)2201.
[5] C. Brites, P. Lima, N.J.O. Silva, A. Millán, V. Amaral, F. Palacio and L. Carlos. Adv. Mater. **22**(2010)4499.
[6] H. Amiri, P. Arosio, M. Corti, A. Lascialfari, R. Bustamante, A. Millán, N.J.O. Silva and F. Palacio, Magn. Res. in Medicine, **66**, 1715-1721, (2011)
[7] Lamiaa M.A. Ali, M. Gutiérrez, R. Cornudella, J.A. Moreno, R. Piñol, L. Gabilondo, A. Millán and F. Palacio, J. Biomed. Nanotechn. (in press) (2013).
[8] O. Haraldseth, R.A. Jones, T.B. Müller, A.K. Fahlvik, A.N. Oksendal, J Magn Reson Imaging, **6**(1996)714.

Figures

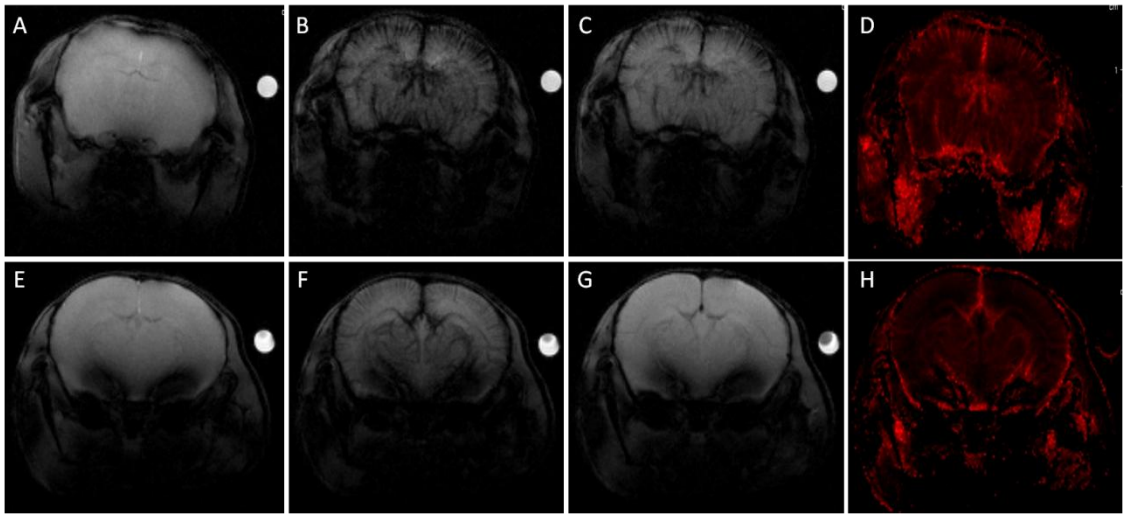

Fig.1. T_2*-weighted images for a mouse brain before (A, E), 5 min (B, F), and 2h (C, G) after injection of Endorem® (upper panel) and ferrofluids (lower panel). rCBV maps for ferrofluids and Endorem® are shown in D and H respectively.

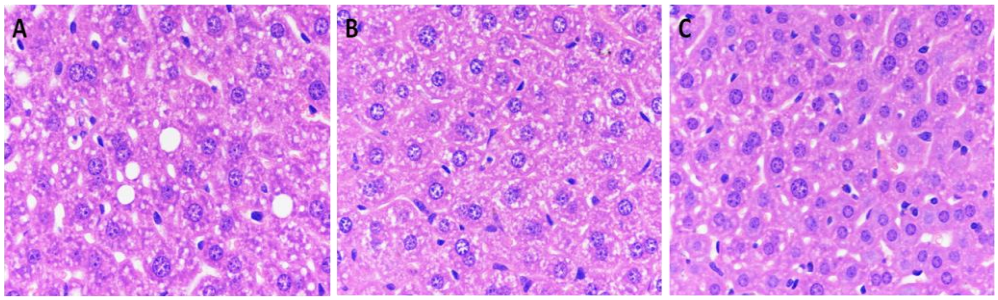

Fig.2. Hematoxylin and Eosin staining in mouse liver before injection (A) and 7 days after injection with Endorem® (B) and ferrofluids (C). Images are at 40X magnifications.