Glucan Particles as a new platform for MRI visualization of inflammatory process

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Introduction

Magnetic resonance is an imaging technique that holds different and well known advantages such as the excellent spatial resolution, the high penetration depth and the absence of ionizing radiations. However, the low sensitivity associated with this technique has led in the last twenty years to the continuous search for low toxic carriers able to allow the delivery to a selected biological target of a high number of diagnostic chemicals. In this wake, Glucan Particles (GPs) have been proposed as novel biocompatible microsystems[1], able to carry into their inner core a huge amount of amphiphilic molecules. Moreover, these yeast-derived particles are easily recognized and taken up by different phenotypes of immune system cells through dectin-1 receptor[2], thus opening the way to the possibility of monitoring inflammatory diseases by MRI. In this work, we loaded GPs with either ^{19}F-containing molecules or paramagnetic Gd-based complexes to investigate the MRI potential applications of these innovative carriers.

Methods

GPs were obtained from common baker's yeast Saccharomyces Cerevisiae after a process of chemical extraction that depletes yeasts of mannan, proteins and lipids, leaving just a leaky shell mainly composed of 1,3-β-D-Glucan. GPs were incubated overnight with either perfluorocarbons (PFCs) or a chloroform solution containing the amphiphilic paramagnetic compound. The day after, PFCs were entrapped inside the particles in form of emulsion through the addition of a polar solvent containing a surfactant, while the amphiphiles were stably trapped just by exploiting a change in solvent polarity. Then, non-entrapped molecules were separated by successive centrifugation-washing cycles. The loaded GPs were characterized in terms of relaxivity and fluorine concentration. The limit of detection for each preparation was determined at 7 T. Moreover, J774.A1 cells were incubated for 1h30 with ^{19}F or Gd loaded GPs, and then diluted with unlabeled macrophages to find the minimum number of macrophages detectable by ^{1}H- and ^{19}F-MRI at 7 T.

Results

Results obtained demonstrated very high values of ^{1}H-relaxivity at 20 MHz for paramagnetic GPs, with an outstanding relaxivity of the system per particle concentration, ranking between 7.7×10^{8} and 2.2×10^{9} $mM_{Gd}^{-1}\cdot s^{-1}$, according to the Gd-compound loaded in the inner core of GPs. Also fluorine concentration obtained in GPs showed a good yield of internalization and looked promising for ^{19}F MRI scanning. All the preparations proved to be stable for at least one month. Dilutions of the obtained microcarriers at different concentrations displayed the possibility of detecting by ^{1}H-MRI 1.0×10^{5} paramagnetic particles/μl, while by ^{19}F-MRI 4.5×10^{5} particles/μl were still detectable (Fig.1). The lower sensitivity of fluorine loaded GPs is balanced by the absence of any background in ^{19}F MRI in comparison to ^{1}H MRI. Moreover, ^{1}H MRI signal is given by the effect exerted on the relaxivity of bulk water by paramagnetic compounds, while ^{19}F signal unequivocally originates from fluorine loaded carriers. Macrophage loading was efficient (Fig.2) and no toxicity was observed. Dilution with unlabeled cells and subsequent MR imaging at 7 T proved the detection of 10% labeled cells both by ^{19}F-MRI and ^{1}H-MRI (Fig.3).

Conclusions

In conclusion, GPs proved to be highly efficient microcarriers for MRI agents, suitable for both ^{1}H and ^{19}F imaging. As *in vitro* results were promising, in the next future possible applications to *in vivo* models of inflammation will be explored.

Acknowledgements

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References

[1] E.R.Soto and G.R.Ostroff. *Bioconjugate Chem.* 19, 840–848 (2008)

[2] S. Figueiredo, J.N. Moreira, C.F. Geraldes, S. Rizzitelli, S.Aime and E. Terreno *Chem. Commun.* 47, 10635–10637 (2011)

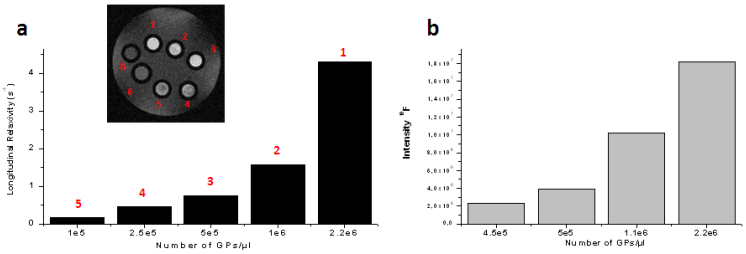

Fig.1: Different dilutions of GPs loaded with a (a) Gd MRI probe, (b) ^{19}F MRI probe. Phantoms were imaged at 7T.

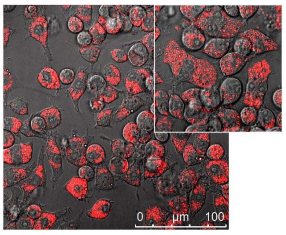

Fig.2: Confocal laser scanning microscopy of J774.A1 cells incubated for 1h30 with Rhodamine DOPE and PFCs loaded GPs.

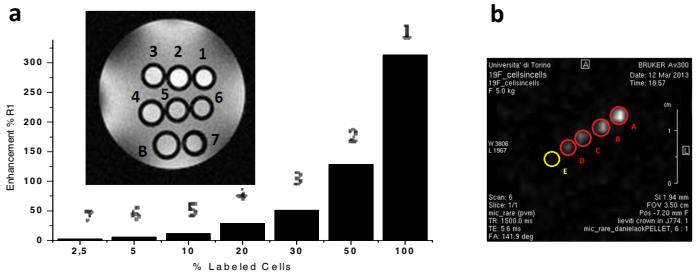

Fig.3: a) ^{1}H MRI of J774.A1 incubated with Gd loaded GPs and diluted to different concentrations (from 100% to 2.5%) with unlabeled cells. b) ^{19}F MRI of J774.A1 incubated with Gd loaded GPs and diluted to different concentrations (A= 50%, B= 30%, C= 20%, D= 10%, E=5%) with unlabeled cells. Phantoms were imaged at 7T.