Effect of Nanoparticle-Protein Interaction on Relaxivity and MRI Contrast Efficiency of Superparamagnetic Iron Oxide Nanoparticles

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Target audience – Biochemists, Biophysicists, and scientists interested in biomedical applications of magnetic nanoparticles.

Purpose – In a biological fluid, proteins associate in a preferential manner with nanoparticles (NPs). The small sizes and high curvature angles of NPs influence the types and the amounts of proteins presented at their surfaces. We were aiming to study the effects of adsorption of a protein corona (PC) on magnetic properties of NPs with different engineered surfaces. This would provide better understanding of possible changes in NP characteristics *in vivo* compared to *in vitro*.

Methods – Two series of superparamagnetic nanoparticles (SPIONs) with different size and functional groups were synthesized with either a single (S-series) or a double (D-series) dextran coating having different surface chemistries/charges, i.e. Plain (-OH), Negative (-COOH) and Positive (-NH_2). The morphologies of various SPIONs were analyzed by transmission electron microscopy (TEM) operating at 200 kV. Zeta potential determination was performed using a Malvern Zetasizer 3000HSa. Compounds magnetization was measured as a function of both temperature (so called zero-field-cooling and field cooling, ZFC/FC) and applied magnetic field (so called hysteresis) using an MPMS-XL superconductor quantum interface (SQUID) magnetometer from Quantum Design. The ZFC/FC experiments were performed at 0.01 T, while the hysteresis plots were obtained at 5K. ^{1}H relaxometry technique was used to obtain the longitudinal, r_1, and transverse, r_2, relaxivities of the SPIONs as a function of Larmor frequency. For frequencies in the range 10 kHz<f<10 MHz, the data were collected with a Smartracer Stelar relaxometer using the Fast-Field-Cycling technique, while for the frequencies f>10 MHz, Stelar Spinmaster and Apollo-Tecmag spectrometers were employed. To obtain T_1 and T_2 values, saturation recovery and CPMG-like sequences were applied, respectively. MRI experiments were performed at 8.5 MHz employing an Artoscan Imager from Esaote S.p.A. The images were obtained scanning the vials containing SPION samples and a well-known MRI contrast agent called Endorem (plain dextran coated SPIONs, not available any more. High resolution spin echo sequence was used with the following parameters: TR/TE/NEX=3s/18ms/1, FOV=180×180, matrix=256×192. All experiments were performed before and after one hour incubation with serum proteins (PBS and 10% Fetal Bovine Serum (FBS) at 37 °C).

Results – Physico-chemical description of the various particles in de-ionized (DI) water is presented in Table 1. The magnetization of the SPIONs decreased in the presence of the protein corona (data not shown). Blocking temperature T_B, the temperature corresponding to the peak in the ZFC curve below which the spins are frozen, is decreased for the D-Plain SPIONs compared to the S-Plain ones, although a slight increase in the presence of the protein corona, relative to the value in the absence of proteins, is seen in both D- and S-Plain samples. The magnetization increases with magnetic field and saturates at a certain field, which is the typical behavior for superparamagnetic systems. Coercive field, and the remanance magnetization of the D-Plain SPION samples are lower than those having a single coating, i.e. S-Plain sample, while the saturation magnetization of the S-Plain SPIONs is slightly lower than the D-Plain ones in the presence of the PC. Fig 1 represents relaxivity data. Longitudinal relaxivity of the SPIONs presents the typical behaviour of superparamagnetic contrast agents. All samples demonstrated transverse relaxivities comparable with, or better than Endorem. The S-Plain and Endorem presented the same relaxivity values, as expected, while the relaxivity value was smaller for the D-Plain (with a bigger hydrodynamic size). Furthermore, D-Positive and S-Negative SPIONs presented relaxivity values higher than that of Endorem. Presence of the PC slightly increased the relaxivity of the negatively charged SPIONs while it dramatically decreased the relaxivity in the case of the positively charged SPIONs. Fig 2a shows an image of the SPION samples before any interaction with the proteins. Figs 2b and 3c present images of S- and D-series SPIONs, respectively, before and after the formation of the PCs. results are in full agreement with the NMR findings.

Discussion –Surface properties of magnetic nanoparticles play important roles in their magnetic properties and the efficiency of MRI contrast enhancement[1]. In our study, the PC was found to affect the magnetic properties and the MRI contrast efficiency of the SPIONs to different extents depending on their different PC compositions. The carboxylate-amine coupling in the negatively charged dextran coating of the SPIONs does not affect the relaxivity, while the amine-carboxylate coupling in the positively charged SPIONs decreases the relaxivity significantly. In other words, protein adsorption to the carboxylate-amine coupling, i.e. NPs functionalized with –COO^- groups, does not affect the relaxivity of the SPIONs, while protein adsorption to the amine-carboxylate coupling in the positively charged SPIONs, i.e. SPIONs functionalized with –NH_3^+ groups, decreases the relaxivity significantly despite the fact that protein absorption to the surface of positive SPIONs is significantly lower than negative SPIONs. **Conclusion –** The results suggest that nanoparticle-protein interactions should be taken into account prior to the development and optimization of multifunctional targeting SPION systems for biomedical applications.

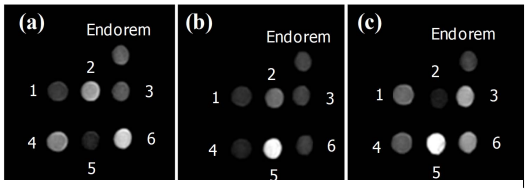

Fig. 2 MRI images of SPIONs (a) samples before incubation with protein; 1: S-Negative, 2: S-Positive, 3: S-Plain, 4: D-Negative, 5: D-Positive, and 6: D-Plain; (b) S-series with and without associated proteins; 1: S-Negative, 2: S-Positive, 3: S-Plain, 4: S-Negative-PC, 5: S-Positive-PC, and 6: S-Plain-PC; (c) D-series with and without associated proteins; 1: D-Negative, 2: D-Positive, 3: D-Plain, 4: D-Negative-PC, 5: D-Positive-PC, 6: D-Plain-PC.

References – [1] J. Huang, X. Zhong, L. Wang, L. Yang, H. Mao, *Theranostics* **2012**, *2*, 86.

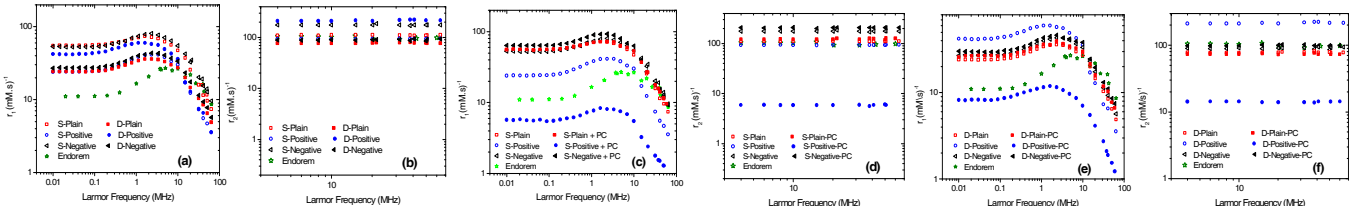

Fig. 1 r_1 and r_2 relaxivities versus frequency for (a, b) S- and D-series, (c, d) S-series and S-series-PC, (e, f) D-series and D-series-PC, respectively.

Table 1. Physico-chemical description of the various particles in DI water.

Sample	Functional group	TEM Size (nm)	D_H (nm)[a]	Zeta-Potential (mV)	Nick-Name
Bare SPION (No Coating)	OH	5±0.5	---	---	Bare
Single Dextran Coated SPION	COOH	15.2±1.2	39.7±0.1	-21.4±0.4	S-Negative
	Plain		74.0±1.0	-9.1±0.7	S-Plain
	NH_2		105.1±6.2	+19.3±0.5	S-Positive
Double Dextran Coated SPION	COOH	[d]	64.6±1.3	-17.8±0.3	D-Negative
	Plain		78.6±0.81	-6.2±0.9	D-Plain
	NH_2		179.5±3.02	+9.3±1.1	D-Positive

[a]z-average hydrodynamic diameter extracted by cumulant analysis of the data.