Effect of charge and coating on superparamagnetic iron oxide nanoparticles (SPION) proteins interactions: in vitro and biodistribution studies

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Introduction

Superparamagnetic iron oxide nanoparticles (SPION) have become important for various *in vivo* and *in vitro* biomedical applications such as imaging, magnetic separation, biosensor devices and therapy.
To be used in biomedical applications, SPION are usually stabilized in physiological media with biocompatible surface coating [1] which could be used for specific targeting or detection [2]. It is commonly observed, *in vivo*, that the SPION are taken up by liver, spleen and the reticulo-endothelial system (RES) a few minutes after injection. Many studies have revealed that the chemical composition of the SPION surface, its charge or size influence biodistribution. However, studying SPION biological interactions, especially with body fluids proteins, is more important and a main challenge to understand their in vivo behavior.

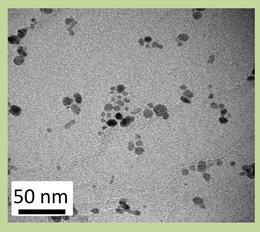
In this study, maghemite SPION (γ-Fe_2O_3) were surface modified with differently charged (positive, neutral and negative) polyvinyl alcohol (PVA) polymers. PVA was used to prevent agglomeration and improve biocompatibility of the magnetic nanoparticles [2]. Surface modified SPION were then characterized with classical methods (crystallite's and hydrodynamic mean diameters and Zeta potential) before incubation with biological media. *In vivo* studies were performed in the rat. The SPION were then injected, in the same conditions, for 15 minutes before sacrificing the animals. The SPION were removed from the rat's blood and the protein distribution was determined in 10 different organs and in the blood.

Materials and Methods

Naked SPION preparation

Iron oxide nanoparticles (SPION) are synthesized by co-precipitation method from mixed solution of $FeCl_2$ and $FeCl_3$ (molar ratio [1:2]) upon the addition of a base and resulting, after controlled oxidation step, in stable maghemite γ-Fe_2O_3 particles.[3]

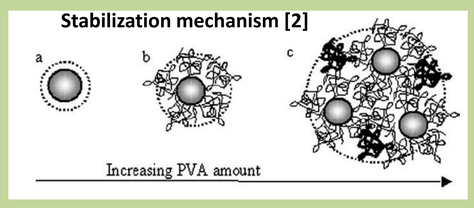
Stable acidic suspension of SPION (**10 mg_{Fe}/mL**)

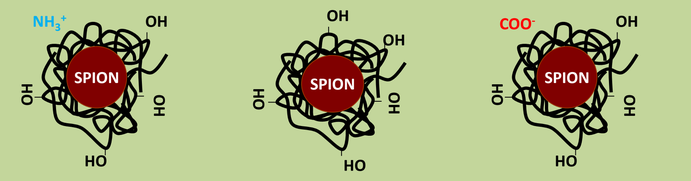


Properties of naked SPION [1]
Crystallites ≈ 9 nm
Aggregates ≈ 25 nm
Zeta Potential at pH 7 ≈ 0mV

Polymer coated SPION (PVA-SPION) preparation

SPION are coated with Polyvinyl alcohol (PVA) polymer with different functional groups (R) to prevent their aggregation and sedimentation in high ionic strength medium [2]





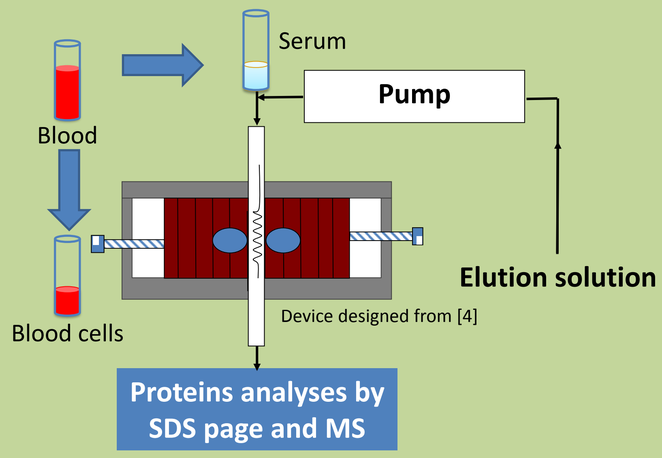
Biological experiments

Female rats (3 rats per condition) were injected either with NaCl solution at 0.15 M (3 control rats) or with 7 mg_{Fe} PVA-SPION. 15 minutes after injections, the rats were sacrificed. The blood and organs were collected for protein adsorption and biodistribution studies.

PVA-SPION with different –R group	dH	Zeta Potential
-NH_2	47 nm	+14 ± 1 mv
-OH	113 nm	+5 ± 1 mV
-COOH	79 nm	-16 ± 1 mV

Proteins interactions on SPION

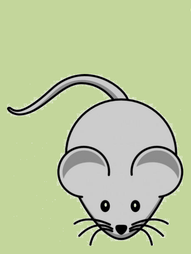
Blood was removed from sacrificed rats and centrifuged in order to study serum. Serum was loaded in a magnetic reactor to elute proteins adsorbed onto PVA-SPION.



SPION biodistribution

Organs were removed and SPION concentration were measured on each organ. The amount of SPION (in mg Fe) was plotted in percentage of SPION injected (7 mg Fe):

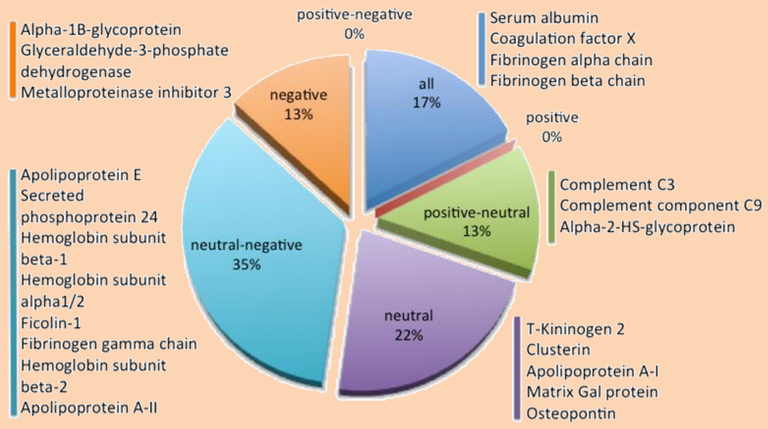
% $SPION_{organ}$ = mg Fe_{organ} / 7 mgFe x 100



- Brain
- Heart
- Lung
- Thymus
- Liver
- Spleen
- Stomach
- Intestine
- Kidney
- Bladder
- Urine
- Blood cells
- Serum

Results

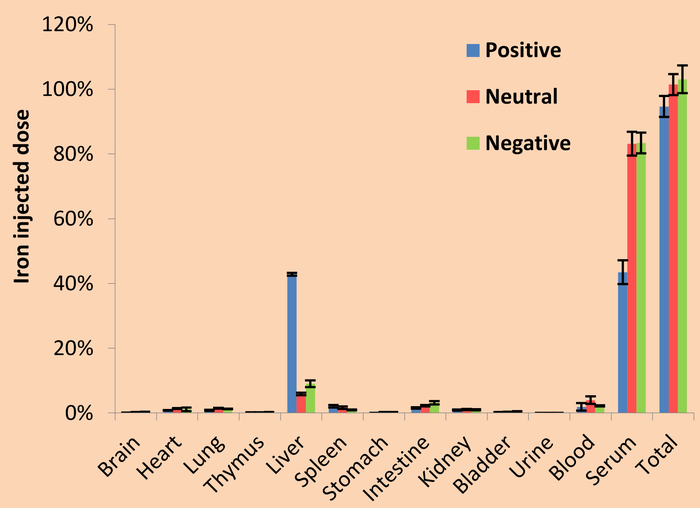
MS analyses of *in vivo* adsorbed proteins



It was possible to trapped SPION after removing from rats blood stream. Moreover the proteins adsorbed on their surface were removed and analyzed. First of all, some proteins were found on all type of PVA-SPION such as albumin, the most abundant blood protein.
More than one third of the proteins adsorbed are shared between neutral and negatively charged SPION when only 13% of them were present on positively and neutral SPION.
It seems that, *in vivo*, the behaviors of neutral and negative PVA-SPION are more similar than the positive PVA-SPION.

Biodistribution of different PVA-SPION

It was possible to recover the injected dose of PVA-SPION from the organs analyses.
15 minutes after injection, already 40% of the positive PVA-SPION were already uptaken by the liver when more than 80% of the Neutral and negative PVA-SPION were still in the blood stream, especially in the serum.
As already observed in proteins adsorption, the behaviors of neutral and negatively charged PVA-SPION are identical and different from the positively charged PVA-SPION.



Conclusion

Focusing *in vivo* behaviors of nanoparticles only as initial charge or size dependence is not so obvious. We showed that, when the absolute charges are not so different, such as for positive and neutral PVA-SPION at respectively +14 and +5 mV, their hepatic uptake are not alike. It looks like that the biodistribution are strongly correlated to their proteins absorption. Moreover, the type of proteins really influence the stealthiness of the magnetic nanoparticles and a deeper analysis of all of them would permit a better understanding of opsonization process for disease nano-targeting.

Acknowledgements

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References: [1] Neuberger, T. *et al.* Magn. Magn. Mater. 2005, 293, 483–496; [2] Petri-Fink, A. *et al.* Biomaterials 2005, 26, 2685–2694 ; [3] Chastellain, M. *et al.* Journal of colloid and interface science 278-2 (2004) 353-360 ; [4] Salaklang, J Thesis EPFL 4539 (2009)