Iron Oxide Nanoparticles (SPION): a Multifunctional Tool for Medical Applications

Lionel Maurizi, Usawadee Sakulkhu, Vianney J. P. Bernau, Géraldine Coullerez, Heinrich Hofmann

Introduction

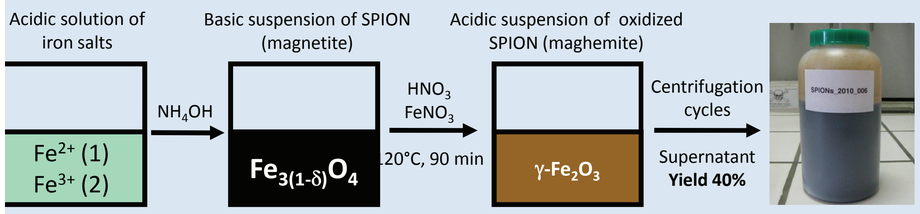
The development of new methods and tools for the targeting and identification of specific biomolecular interactions within living systems is of great interest in the fields of systems biology, target and drug identification, drug delivery, and diagnostics. Super-Paramagnetic Iron Oxide Nanoparticles (SPION) show very interesting and novel combination of properties when compared to other nanoparticles, depending on their inducible magnetization allowing them to be directed in a defined location combining biological ligand and an external magnetic field. The SPION can be used in medical applications such as MRI contrast agents and as local heat source in the case of tumor therapy (hyperthermia).

The rapid clearance of SPION from the blood stream is one of the major challenges for their *in vivo* biomedical application. Surface coatings are used to control over interfacial chemistry, which is thought to influence plasma protein adsorption and particle aggregation. The synthesis of core-shell magnetic nanoparticles includes: (i) SPION synthesis, (ii) core-shell structures with organic or inorganic biopassive coating, (iii) attachment of bioligands.

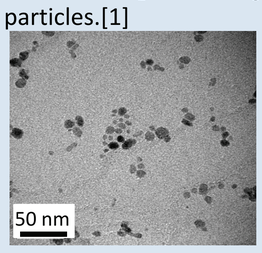
SPION synthesis and surface modification

Naked SPION

Iron oxide nanoparticles 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 in stable maghemite γ-Fe_2O_3 particles.[1]

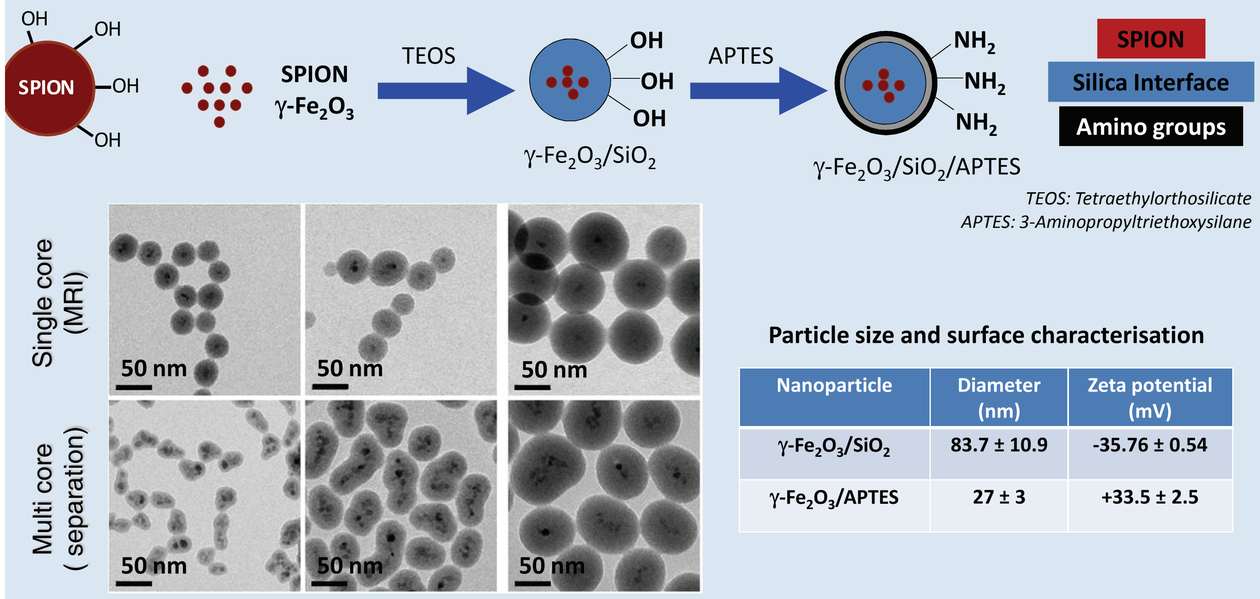


Stable acidic suspension of SPION (**10 mg_{Fe}/mL**)



Properties of naked SPION [2]
Crystallites ≈ 9 nm
Aggregates ≈ 25 nm
Zeta Potential at pH 7 ≈ 0mV

Silica coated SPION

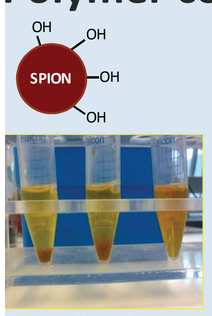


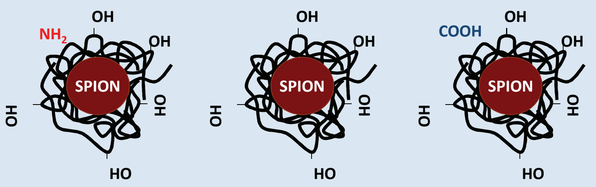
TEOS: Tetraethylorthosilicate
APTES: 3-Aminopropyltriethoxysilane

Particle size and surface characterisation

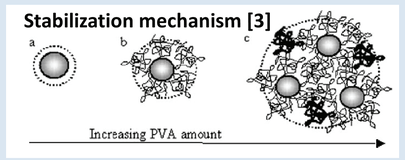
Nanoparticle	Diameter (nm)	Zeta potential (mV)
γ-Fe_2O_3/SiO_2	83.7 ± 10.9	-35.76 ± 0.54
γ-Fe_2O_3/APTES	27 ± 3	+33.5 ± 2.5

Polymer coated SPION





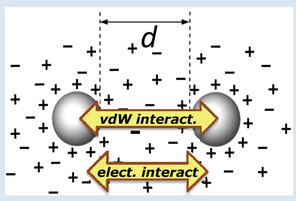
Amino-PVA 43.34 0.91 mV **Positive**
PVA-OH 9.28 0.75 mV **Neutral**
PVA-**COOH** -15.92 0.56 mV **Negative**



Understanding of colloidal SPION behavior

A classical way to predict particle-particle interactions in a suspension is done by use of the Derjaguin-Landau and Verwey-Overbeek (DLVO) theory [4] [5]. This theory has been repeatedly shown to predict with good agreement with experiments the forces of interactions for particles typically above 100 nm in simple, dilute solutions ($c_{ionic} \approx 10^{-2}$ M).

$$\Phi_{DLVO} = \Phi_{vdW} + \Phi_{es}$$



Inspired from Velegol's [6] work (among others), we propose for the case of this project to investigate the suitability of an **well adapted, extended DLVO theory** as follow:

$$\Phi_{X-DLVO} = \Phi_{wdV} + \Phi_{es} + \Phi_{dep} + \Phi_{steric} + \Phi_{bio} + \Phi_{magn}$$

With
Φ_{vdW} **London-van der Waals interaction:** Coupling of the spontaneous momentary dipolar of moments of the particles (always positive)
Φ_{es} **Electrostatic interaction:** Coulombic repulsion between two same sign charged particles
Φ_{dep} **Depletion interaction:** Forces resulting from osmotic pressure, which arise in presence of solvent molecules
Φ_{steric} **Steric interaction:** When soft biomolecules are coating the particles surfaces, it can act as a steric barrier (could prevent strong VdW interactions)
Φ_{bio} **Biological interaction:** Very complex interactions arise between particles and complex bio-fluids
Φ_{magn} **Magnetic interaction:** Attractive magnetic dipole-dipole interactions arise between the particles when put into a magnetic field

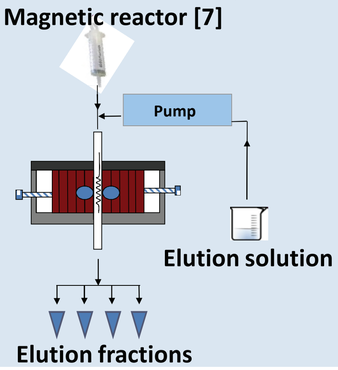
Based on this simple equation, we wish to bring understanding whether the physical models describing the different potentials

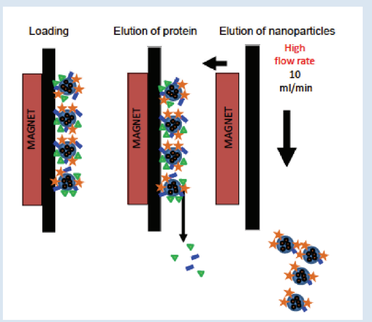
- are relevant / dominant in our particle system,
- are developed from assumptions (physically "valid") for both nanosized particles *and* complex suspending media
- are in good agreement with experimental results

Fixed bed magnetic reactor for coating of SPION and protein separation

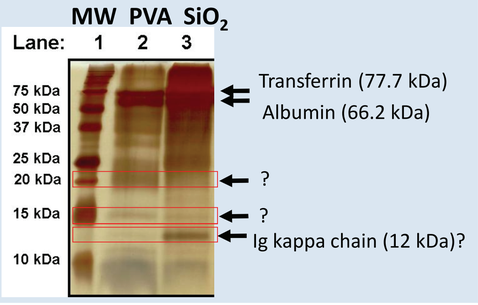
A fixed-bed magnetic reactor is used for eluting proteins adsorbed on SPION and for their identification with electrophoresis. A device with multiple magnets is under development to coat and functionalize SPION in continuous and in more reproducible way.

Magnetic reactor [7]



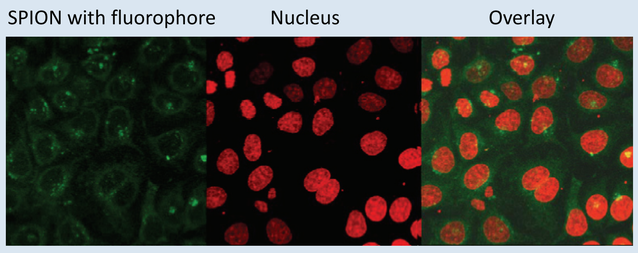


SDS-PAGE
Sodium dodecyl sulfate polyacrylamide gel electrophoresis



Medical applications of SPION

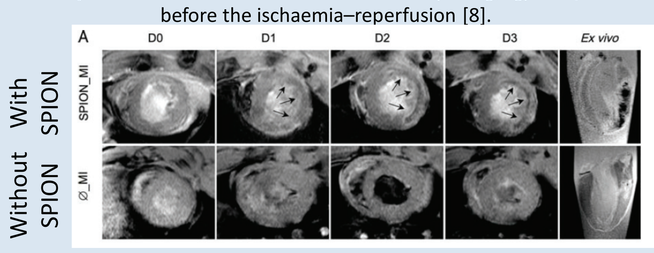
In-vitro internalization



Confocal fluorescence microscopy of SPION functionalized with a fluorophore (FITC) and incubated with HeLa cells: a) green fluorescence of functionalized SPION, b) the nucleii of the cell colored in red by Hoechs, c) the overlay of the two first pictures showing the internalization of these SPION inside HeLa cells but not in the nucleus

In-vivo Monocyte Targeting (MRI)

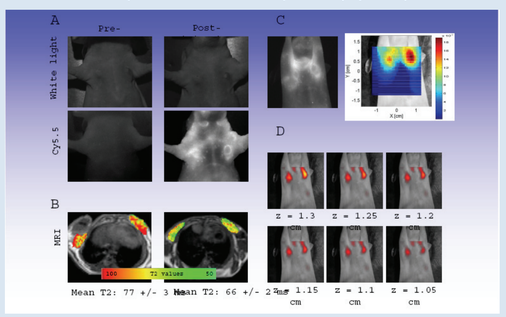
Injection of fluorescent PVA coated SPION (10 mg/kg) 3 days before the ischaemia–reperfusion [8].



In vivo magnetic resonance imaging of infarcted groups of rats. The first line corresponds to a representative rats group injected with SPION and clearly shows the appearance over time [Day (D) 0 to D3] of a hypointense (black) signal in the myocardial infarction area (arrows). The second line corresponds to a representative control rat group and does not show any hypointense signal.

In-vivo imaging of functionalized SPION

In vivo magnetic anf fluorescent imaging of RGD-SPION coupled with a fluorophore (Cy 5.5)



X .Montet, HUG Genève

Conclusion and outlooks

The size and the surface properties, e.g. zeta potential of SPION were tuned with polymer surfactants or inorganic silica "coating for medical applications such as MRI contrast agents or *in vitro* cellular uptake. Their colloidal behavior is under investigation to better understand the stability of particle suspension in different biological media, e.g. DMEM, RPMI upon ionic strength and proteins adsorption. However, better optimization of synthesis parameters would permit to elaborate nanoparticles with surface properties suitable for a range of biomedical application as contrast agent for imaging or cell delivery of therapeutic drugs. Thanks to the magnetic fixed-bed reactor it will be possible to control the coupling of proteins or antibody to SPION for specific detection *in vitro* and *in vivo*.

Publications

[1] Massart, R. Ieee Transactions on Magnetics 17 (2), 1247 (1981); [2] Chastellain M. 2004. Nanoscale superparamagnetic composite particles for biomedical applications. EPFL Thesis n°3045; [3] Petri-Fink A. et al. European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 129–137; [4] B. Derjaguin and L. Landau, Acta Physicochimica U.R.S.S, vol. 14, no. 6, 1941; [5] E. J. W. Verwey and J. T. G. Overbeek, Elsevier publishing company, inc., 1948; [6] D. Velegol, Journal of Nanophotonics, vol. 1, pp. 012502-25, Jun. 2007; [7] Salaklang J. 2009. Advanced surface derivatization of superparamagnetic iron oxide nanoparticles in a fixed bed magnetic reactor for bio-application. EPFL Thesis n°4539; [8] X. Montet *et al*, European Heart Journal, 2010