Different Surface Superparamagnetic Iron Oxide Nanoparticles and Their Behaviour in Biological Environment

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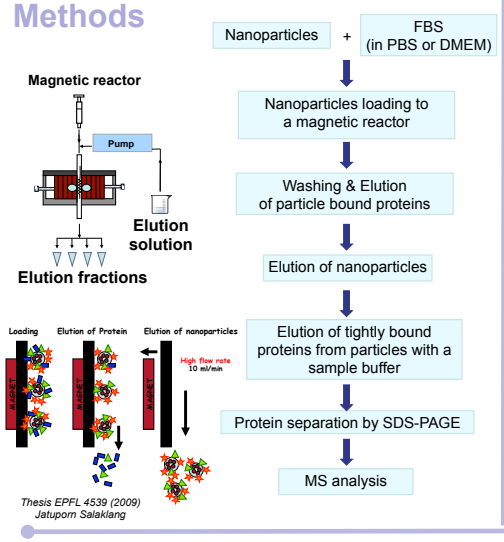
Abstract

Determining of protein compositions on different surface of SPIONs core nanoparticles would facilitate the understanding of cell internalization mechanism of the particles, transport pathways, interaction partners, as well as cellular and molecular function. In this work, we studied the protein profile on nanoparticles of different surface coating, e.g. polymer, inorganic and of different surface charge. The SPION magnetic core is an advantage for easy magnetic separation. Nanoparticle-protein complex are separated from the excess of serum proteins and the adsorbed proteins eluted from the nanoparticle surface using a magnetic fixed bed reactor. The results showed that nanoparticle surface strongly influence the adsorption of serum proteins on nanoparticle surface and nanoparticle behaviour in biological environment, e.g. cell uptake. Nanoparticles, with same surface coatings for instance, silica and shell/core silica/SPIONs nanoparticles showed similar pattern of adsorbed proteins while the different surface materials and different surface charge nanoparticles showed different protein patterns. In addition, this protein separation technique is a part of Nanodiara project for nanoproteomics analysis of biological fluids from Arthritis patients. In addition, these particles will be used for protein separation, diagnostic, bioimaging and biomedical applications.

Methods



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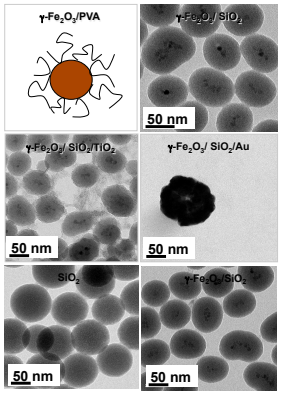
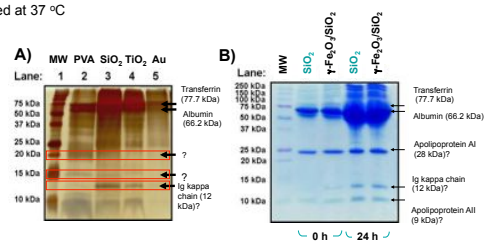
Results

Protein corona on different surface materials

Nanoparticles in DMEM +10% serum incubated at 37 °C





Nanoparticle	Diameter (nm)	Zeta potential (mV)
γ-Fe_2O_3/PVA	~24	43.3 ± 0.9
γ-Fe_2O_3/SiO_2	83.7±10.9	-35.8±0.5
γ-Fe_2O_3/SiO_2/TiO_2	85.7±15.7	-30.9±0.6
γ-Fe_2O_3/SiO_2/Au	143.9±18.4	-16.2±0.9

→ A) after 1h incubation time and magnetic separation (gradient KCl)

→ B) SiO_2 and γ-Fe_2O_3/SiO_2 : 24h incubation time washed with PBS by using centrifugation

1D-SDS PAGE (Preliminary results)

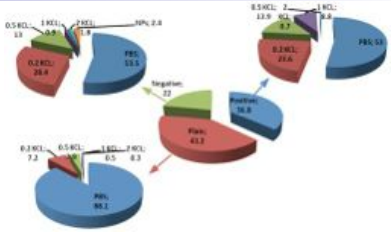
DMEM: Dulbecco's Modified Eagle Medium, PBS: Phosphate buffer saline

Results

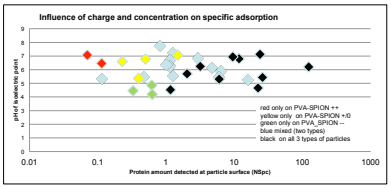
Table 1: MS analysis of protein bound on different surface charged polymer coated nanoparticles

Gel band Mw (Da)	Protein identity (k)	% (NSpC_k/ NSpC_total)	Particle charge
144053	Complement factor H	0.02	
621279	Hemicentin-1	0.04	
39945	AMBP protein	1.58	
43280	Fetuin-B	1.00	
46292	Pigment epithelium-derived factor	0.34	
46300	Endopin-1	0.77	
46351	Alpha-1-antiproteinase	2.02	
139681	Collagen alpha-1(I) chain	0.16	
164325	Alpha-2-macroglobulin	0.04	
22498	Tetranectin	0.13	
39008	Lumican	0.08	
39284	Beta-2-glycoprotein 1	0.30	
86462	Complement factor B	0.50	
106738	Inter-alpha-trypsin inhibitor heavy chain	0.17	
36130	Ecto-ADP-ribosyltransferase 4	0.24	
39038	Alpha-2-HS-glycoprotein	5.30	
51410	Angiotensinogen	0.38	
51875	Factor XIIa inhibitor	0.27	
52728	Antithrombin-III	0.38	
93364	Plasminogen	0.42	
50458	Serine protease inhibitor A3F	0.11	
53182	Vitronectin	0.42	
54915	Vitronectin	0.61	
79571	Testis-specific Y-encoded-like protein 2	0.21	
80521	Fibulin-1	0.21	
23398	Alpha-1-acid glycoprotein	7.50	
30258	Apolipoprotein A-I	1.99	
33980	Alpha-1B-glycoprotein	3.20	
54595	Vitamin D-binding protein	4.57	
54906	Alpha-2-antiplasmin	1.07	
55791	Cytochrome P450 2C5	0.21	
67385	Fibrinogen alpha chain	2.16	
69720	Kininogen-1	3.94	
70015	Alpha-fetoprotein	8.71	
70858	Serum albumin	41.48	
71570	Mutated melanoma-associated antigen 1	0.39	
71610	Prothrombin	0.68	
79450	Serotransferrin	8.02	
186377	Complement C3	0.38	

$$NpSpC\_k = \frac{(SpC/M_w)_k}{\sum_{(n)}(SpC/M_w)_n} \times 100$$



Normalized spectral counts (NSpC) of all associated proteins and their affinities (i.e. dissociation from protein corona composition by washing with various solutions) on the surface of various PVA-coated SPIONs



Proteins eluted from PVA-SPION particles (negative, neutral, positive) and identified by LC-MS/MS (Normalized spectral count (NSpC) values for each protein hit are spectral counts normalized to the total intensity of the protein corona formed on one nanoparticle type).

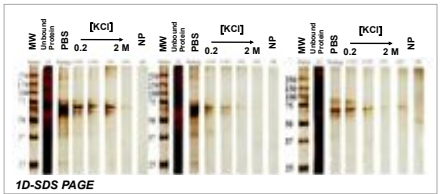
Positive (43.3 mV) | Neutral & Negative | Positive & Negative
Neutral & Positive | Negative (-15.9 mV)
Neutral (9.2mV) | Negative, Neutral & Positive

NSpC_k is the percentage normalized spectral count for protein k, SpC is the spectral count identified, and Mw is the molecular weight in kDa for protein k. This correction takes into account the protein size and evaluates the real contribution of each protein to the hard corona composition. The SpC of each protein was normalized to the protein mass and expressed as relative protein % per PVA coated SPIONs upon the formula (1)

% (NSpC_k / NSpC_total): Percentage of spectral count of detected protein k normalized to the total count of the most abundant proteins identified on PVA-SPION nanoparticles (positive, neutral, negative).

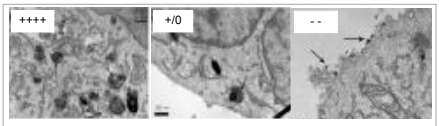
Protein corona on different surface charged PVA-SPION

Fetal bovine serum / particle surface (2.8 ml/m²) incubated at 37 °C for 1h

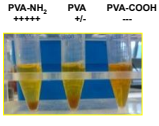


1D-SDS PAGE

PVA-SPIONs	Hydrodynamic Diameter (nm)	Zeta potential (mV)
PVA-NH_2	24	43.34 ± 0.91
PVA (neutral)	30	9.28 ± 0.75
PVA-COOH	47	-15.92 ± 0.56



BioTEM of HeLa cell after incubation with different surface charged nanoparticles



Summary & Outlook

- Surface composition and charge, e.g. positive, neutral and negative resulted in different patterns of adsorbed proteins.
- Different nanoparticle properties may facilitate specific proteins fishing.
- Understanding particles behavior in different biological media (e.g. human serum, urine, synovial fluid) and particle-cell interactions.

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