

Electronic Supplementary Information (ESI)

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gels

The interaction of the different PVA-SPIONs with protein was initially observed via SDS-PAGE.

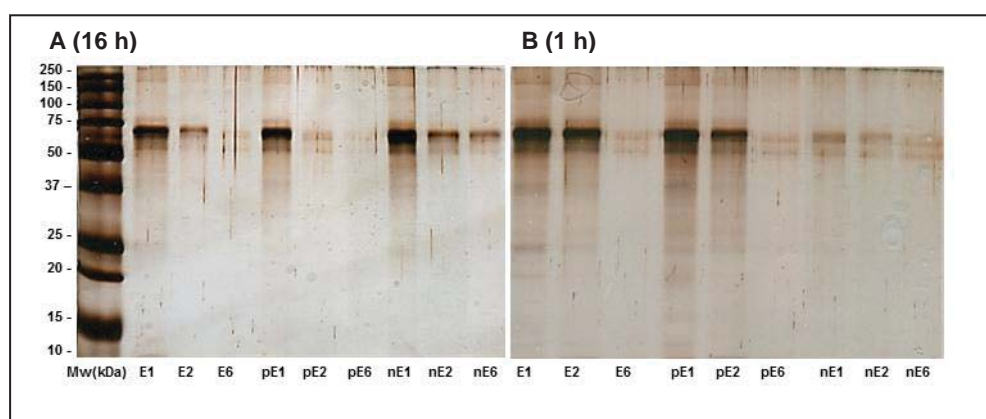


Fig. S1. 1D SDS-PAGE of elution fraction (A) E1 and E2 after 16 h of incubation with 10% FBS supplemented cell culture medium (eluted with 50 mM KCl) and E6 after 16 h of incubation (eluted with 100 mM KCl) and (B) E1 and E2 after 1 h incubation with 10% FBS supplemented cell culture medium (eluted with 50 mM KCl) and E6 after 1 h of incubation (eluted with 100 mM KCl). Proteins were stained with silver nitrate using an adapted protocol to that originally described by Rabilloud *et al*¹.

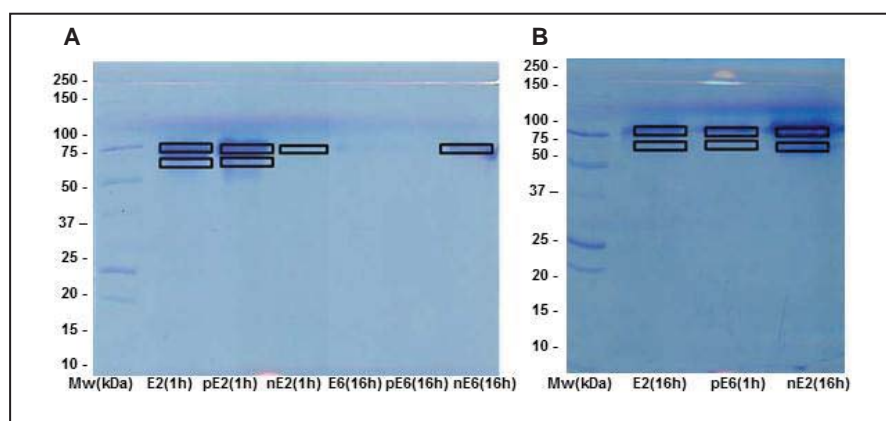


Fig. S2. 1D SDS-PAGE of elution fraction (A) E2 after 1 h incubation with 10% FBS supplemented cell culture medium (eluted with 50 mM KCl) and E6 after 16 h incubation (eluted with 100 mM KCl) and (B) E2 after 16 h incubation (eluted with 50 mM KCl). Black rectangles indicate excised bands used for LC-MS/MS measurements. Proteins were stained with Coomassie Brilliant Blue R-250 Staining Solution (Bio-Rad, Switzerland).

N.B. Ex, pEx and nEx hereby refers to elution fractions eluted from PVA-SPIONs, positive PVA-SPIONs and negative PVA-SPIONs.

Cytotoxicity

Lactate dehydrogenase (LDH) Release

A Cytotoxicity Detection Kit (Roche Applied Science, Germany) was used to quantify the level of cytotoxicity exerted upon the HeLa cells following exposure to PVA-SPIONs. This calorimetric assay was based on the measurement of LDH activity, an enzyme released from the cytosol of damaged cells into the supernatant. Cultivation of HeLa cells was carried out as previously described in the materials and methods section of the main text. Neutral, positive and negative PVA-SPIONs were then exposed at five concentrations (10, 20, 40, 60, 80, and 100 µg/ml Fe all diluted in 10% FBS supplemented cell culture media) for 1, 6, 16 and 24 h to HeLa cells at 37 °C and 5% CO₂. The negative control was 10% FBS supplemented cell culture media only. The detergent Triton X-100 acted as the positive control at a concentration of 0.2% in PBS. Each exposure was repeated a total of four times (n=4). Supernatants were measured in triplicate at 490 nm (with a reference of 630nm) using a multi-plate spectrometer (Benchmark Plus, Bio-Rad, Switzerland). Total extracellular LDH was then measured according to the manufacturer's manual.

LDH Adsorption

The potential for the LDH enzyme to adsorb to the surface of PVA-SPIONs, and therefore result in a false negative cytotoxicity was also assessed for all investigated PVA-SPIONs as previously described by Clift *et al* (2008). No LDH adsorption was observed (data not shown)

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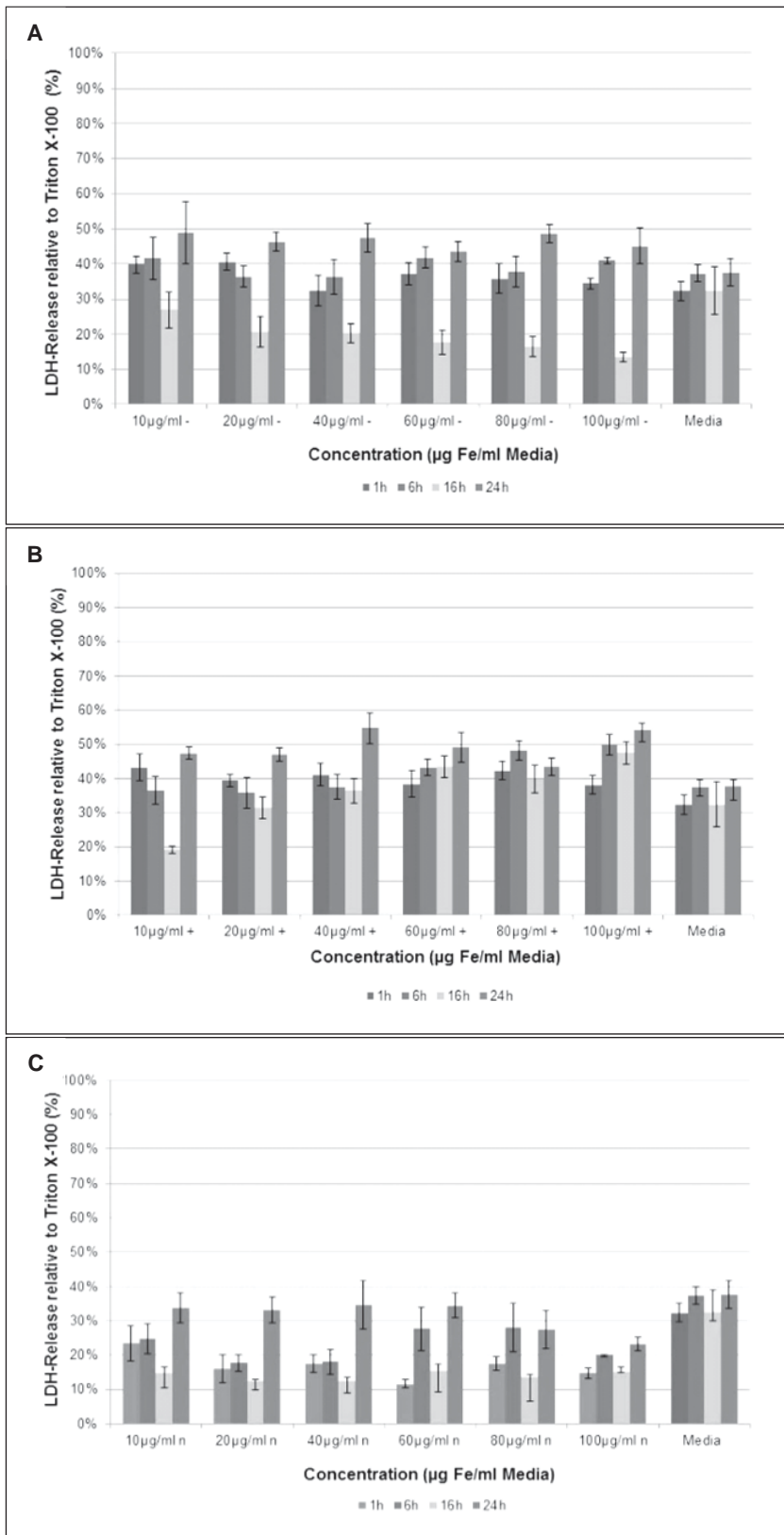


Fig. S3. Release of Lactate Dehydrogenase (LDH) in % by HeLa cells relative to Triton X-100 after 1h, 6h, 16h and 24h as a function of different concentration (µg Fe/ml in supplemented medium) of (A) negatively, (B) positively and (C) neutrally charged P VA-SPIONS. (n=4; Error Bars=SEM)

Concentration Measurements by Ultraviolet/Visible Spectroscopy (UV/Vis)

The colloidal stability of the positive charged PVA-SPION aggregates was assessed by the concentration with UV/Vis (Jasco V-670 UV-Vis_NIR spectrophotometer). Particle suspensions were made to a concentration of 100 $\mu\text{g/ml}$ using cell culture media (DMEM) in presence of 10% FBS and the absorbance of the suspensions was measured at a wavelength of 400 nm as a function of time (readings taken over a 24 h period at 10 min intervals). The samples were sealed in a glass cuvette and maintained at 37°C throughout to mimic cell culture conditions.

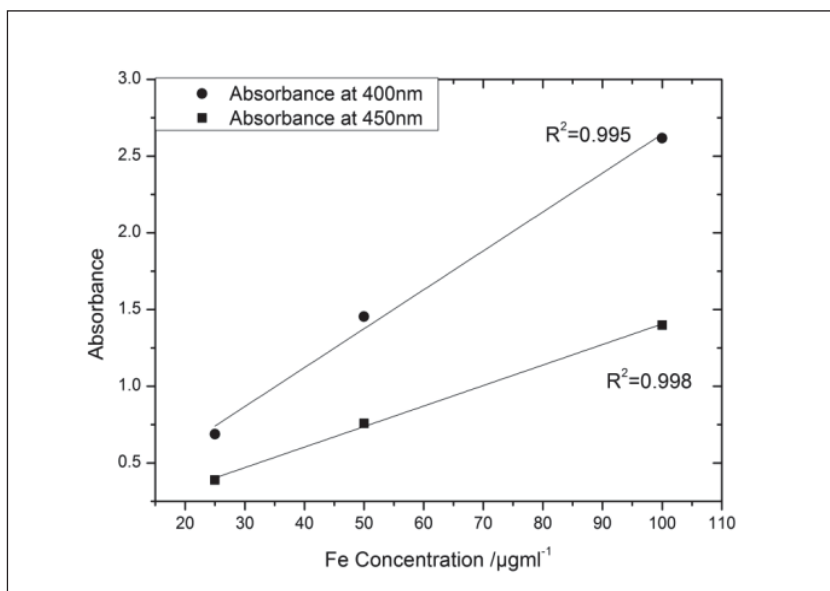


Fig. S4. Standard curve of freshly dispersed p-PVA-SPIONs in serum at two different wavelengths

The two fits gave the relation between absorbance and concentration as shown below.

$$A(400\text{nm}) = 0.0254c_{Fe} + 0.106$$

$$A(450\text{nm}) = 0.0134c_{Fe} + 0.068$$

N.B. The linear fits do not pass through the origin due to a small absorbance at these wavelengths of the media and serum.

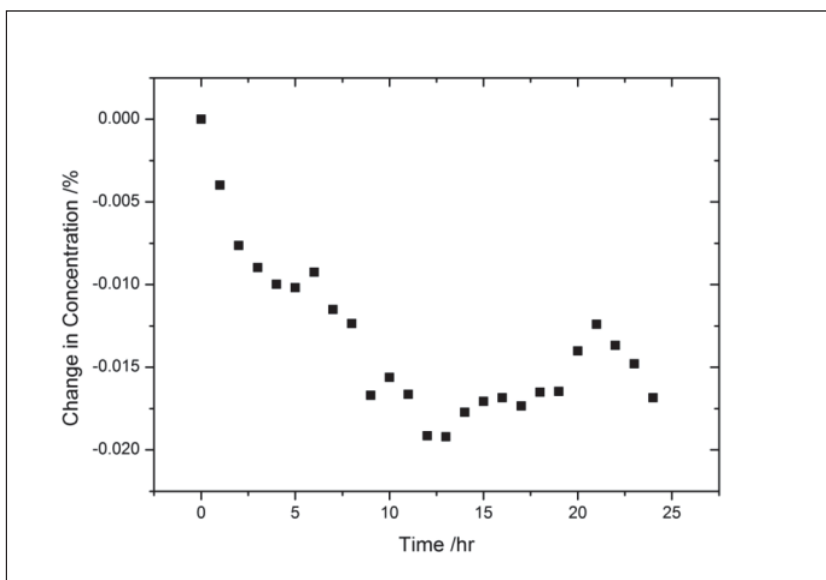


Fig. S5. Change in concentration of Fe of p-PVA-SPIONs with time in serum over time as measured by UV-Vis using the fits in Figure S2 and an absorbance at 450nm.

Calculation of sedimentation parameters

Using equations by Cho *et al* (2011), the following parameters were calculated assuming an aggregate of PVA-SPIONs with a diameter of 500nm, similar to the sizes found by DLS ³.

Table S1. Parameters calculated assuming PVA-SPION aggregation

Parameter	Value	Unit
Particle radius	500	nm
Diffusion Coefficient (D)	4.36×10^{-13}	$m^2 s^{-1}$
Volume of Particle	5.24×10^8	nm^3
Diffusion Velocity (V_D)	0.87	$nm s^{-1}$
Sedimentation Velocity (V_S)	0.76	$nm s^{-1}$
V_S/V_D	0.87	
Density of Particle (aggregate)	1.35×10^{-21}	$g nm^{-3}$
Drag coefficient	4.72×10^{-09}	$g s^{-1}$
Sedimentation Coefficient	4.85×10^{-09}	s

N.B. The values chosen for the particle size consider the extreme case of twice the size of the average radius measured by DLS.

The sedimentation velocities of single PVA-SPIONs were not calculated, as it is known that these do not sediment in aqueous solutions.

References - Electronic Supplementary Information (ESI)

1. M. Swain and N. W. Ross, *Electrophoresis*, 1995, **16**(6), 948.
2. M. J. D. Clift, B. Rothen-Rutishauser, D. M. Brown, R. Duffin, K. Donaldson, L. Proudfoot, K. Guy and V. Stone, *Toxicol. Appl. Pharmacol*, 2008, **232**(3), 418.
3. E. C. Cho, Q. Zhang and Y. Xia, *Nature Nanotech*, 2011, **6**(6), 385.