

Biocompatibility assessment of sub-5 nm silica-coated superparamagnetic iron oxide nanoparticles in human stem cell and in mice for potential application in nanomedicine

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Supplementary Material

Target gene	Primer sequence	Annealing temperature (C°)
VEGF	F 5'-cttgggtgcattggagcct-3' R 5'-ctgcgctgatagacatccat-3'	60
β-ACT	F 5'-gctcctcctgagcgcaag-3' R 5'-catctgctggaaggtggaca-3'	60
Ki67	F 5'-tgaacaaaaggcaaagaagac-3' R 5'-gagctttccctattattatggt-3'	60
IDO	F 5'-tgctaaaggcgctggttgaa-3' R 5'-tacaccagaccgtctgatag-3'	60
HGF	F 5'-caatagcatgtcaagtggag-3' R 5'-ctgtgttcgtgtggtatcat -3'	60
OPN	F 5'-gtgtgggtttatggactgagg-3' R 5'-acgggggatggccttgtatg-3'	60
RUNX2	F 5'-catcatctctgccccctct-3' R 5'-actcttgccctcgtccactc-3'	60
ALP	F 5'-caatgagggcaccgtggg-3' R 5'-tcgtggtggtcacaatgcc-3'	60
OCL	F 5'-gcagcgaggtagtgaagag-3' R 5'-gaaagccgatgtggtcagc-3'	60
PAX7	F 5'-gcaaattgctgtcctgctca-3' R 5'-gaaaactggtcacatctgcct-3'	60
MYOD	F 5'-atgatggactacagcgcc-3' R 5'-agatgcgctccacgatgc-3'	60
MYF5	F 5'-gagagcaggtggagaactac-3' R 5'-gatgctgtcaaaagtactgc-3'	60
Dystrophin	F 5'-cacaaaatgggtaaatgcaca-3' R 5'-ccatcctgtaggtcactgaa-3'	60
GAPDH	F 5'-catcatctctgccccctct-3' R 5'-caaagttgtcatggatgacct-3'	60

Table S1. List of primer sequences used for RT-qPCR analysis in this study. F: Forward primer. R: reverse primer.

Particle	Size in water at each time point (nm±SE)				
	0h	3h	6h	9h	12h
Fe ₃ O ₄	3.6±0.4	3.8±0.58	3.61±0.72	4.12±0.74	4.09±0.20
Fe ₃ O ₄ -SIO	4.6±0.5	4.91±0.70	5.41±0.75	4.9±0.50	4.87±0.47

Table S2. Dynamic Light Scattering characterization of SIO naked nanoparticles after exposure to pure water.

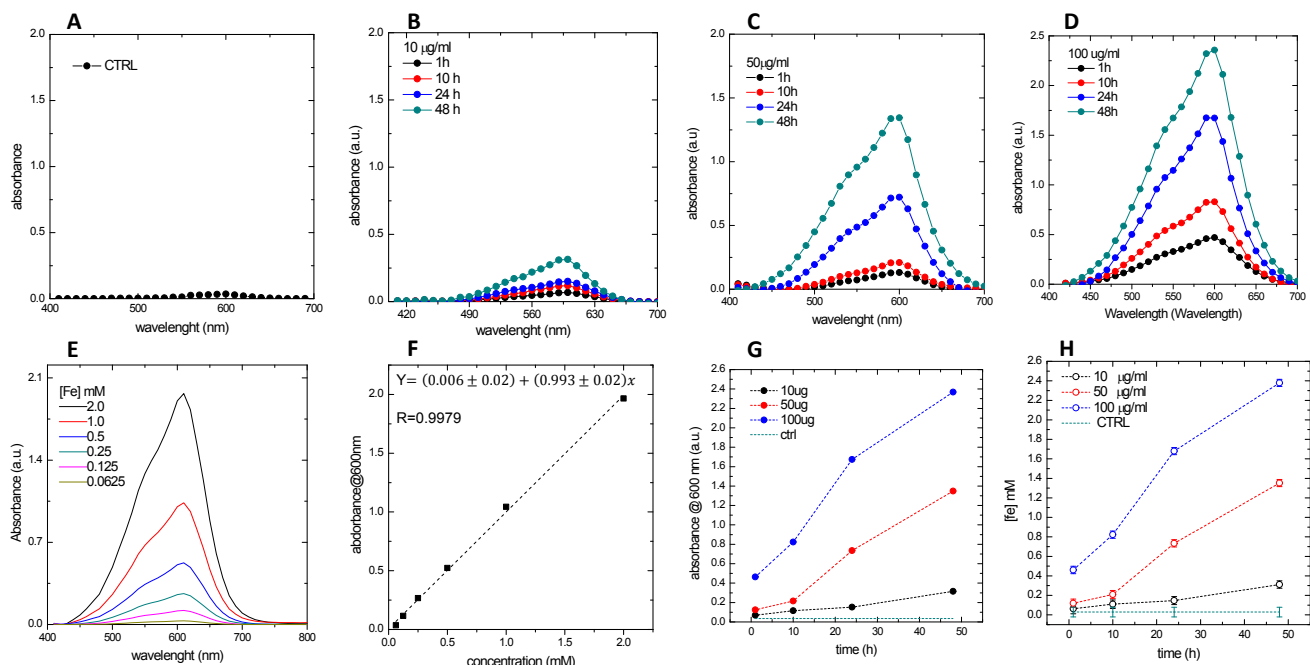


Fig. S1. Blank subtracted absorbance spectra for the control (A) and the treated samples with different NP concentrations, namely 10 $\mu\text{g/ml}$ (B), 50 $\mu\text{g/ml}$ (C) and 100 $\mu\text{g/ml}$ (D) at different incubation times (1h, 10h, 24h, 48h). Blank subtracted absorbance spectra of the iron standards used for the calibration curve in the 0.06 – 2.0 mM concentration range (E). Calibration curve (F) with the corresponding least-squares line fitted to the standard points ($R=0.9979$). Absorbance at $\lambda_{\text{max}}=600$ nm of the blank subtracted samples as a function of the incubation time at different NP concentrations (G). Total Iron concentration in the sample solutions as a function of the incubation time at different NP concentrations (H).

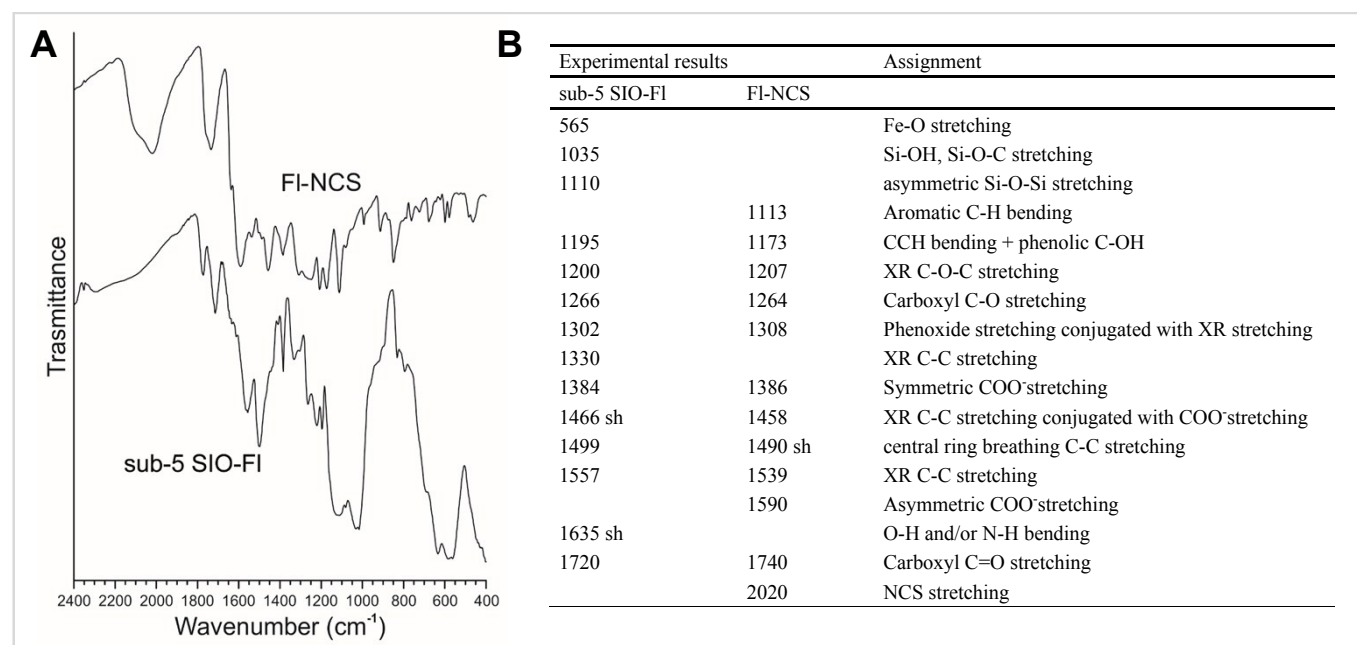


Fig. S2. (A) Fourier transform infrared spectroscopy (FTIR) spectra in the 2400–400 cm^{-1} region of sub-5nm silica-coated magnetic iron oxide nanoparticles (sub-5 SIO-FI) and pristine fluorescein isothiocyanate (FI-NCS). (B) Peak position (cm^{-1}) and assignment for sub-5 SIO-FI and pristine FI-NCS. Sh, shoulder; XR, xanthene ring

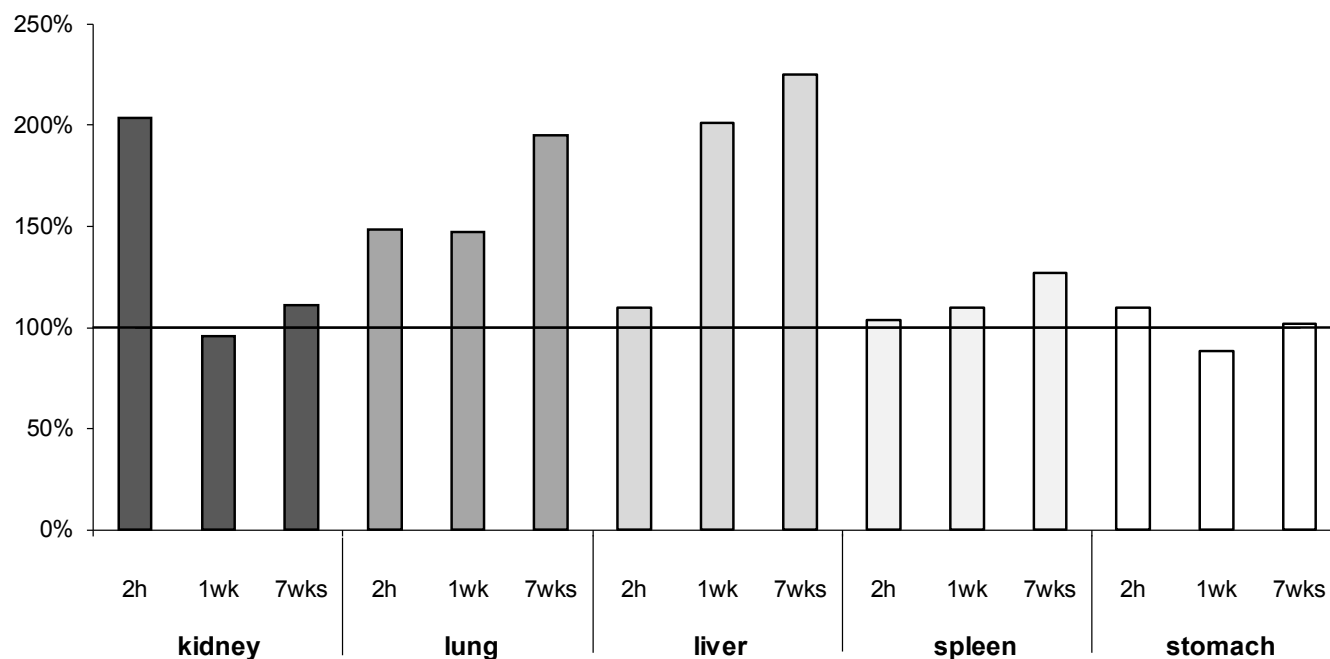


Fig. S3. *In vivo* percentage tissue distribution. Kidney, lung, liver, spleen, stomach organs collected from CD-1 mice 2 hours, 1 week and 7 weeks post-injection of sub-5 SIO-FI nanoparticles (10 mg / kg body weight). Data are reported as percentage relative to the control, indicated by the black line. Mice are grouped per organ type. n=4. * $P < 0.05$ versus the control group.

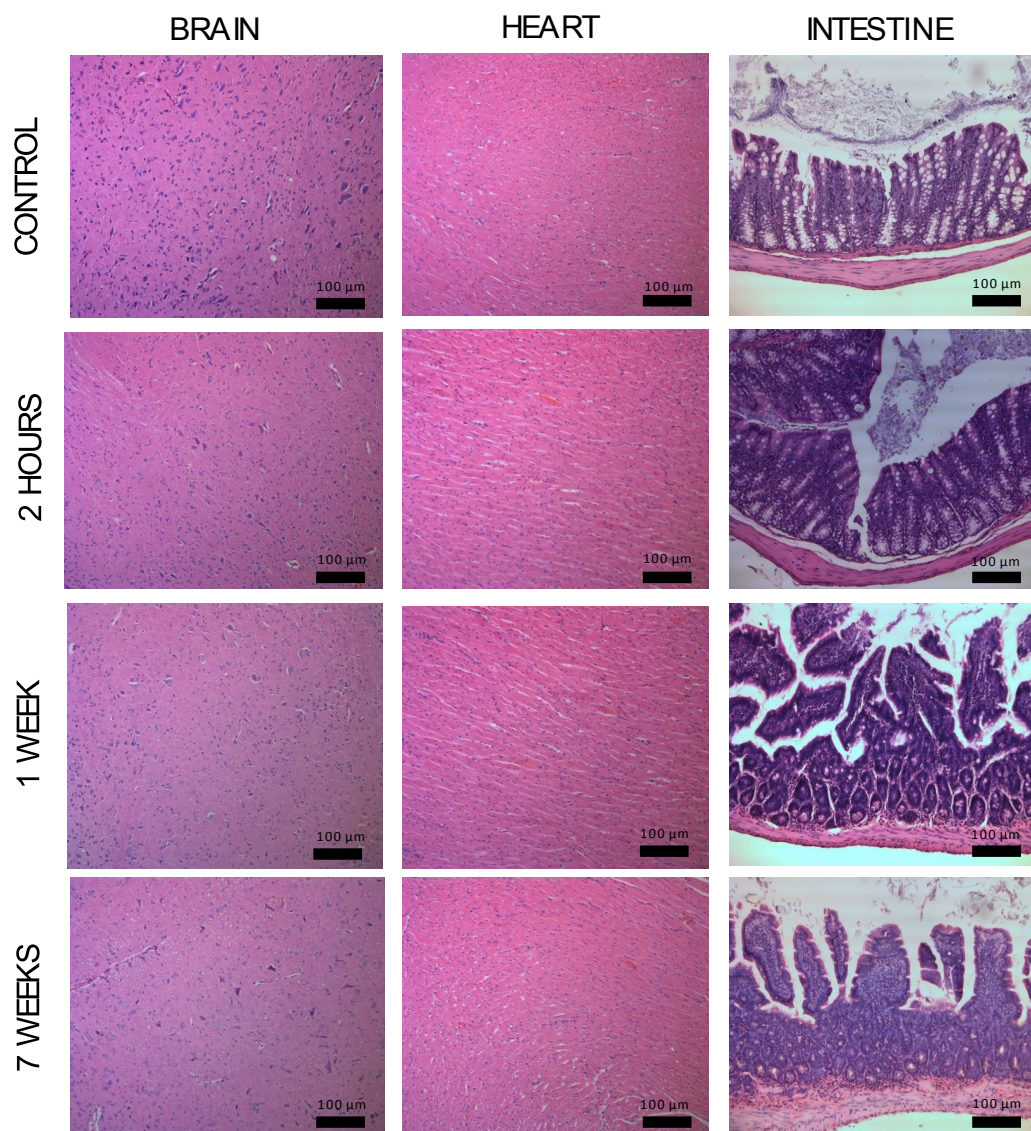


Fig. S4. Histological examination. Representative photographs of H&E stained tissue sections from other organs (brain, heart and intestine). Ten/twelve-week-old female CD-1 mice (48 ± 3 g weight) were treated (4 animals/group) *i.v.* with 10mg/kg of sub-5 SIO-FI nanoparticles. Brain, heart and intestine were examined for potential tissue damage, inflammation or lesions at 2 hours, 1 week and 7 weeks from sub-5 SIO-FI nanoparticle injection, in comparison with untreated animals (control) tissue sections. (10X)

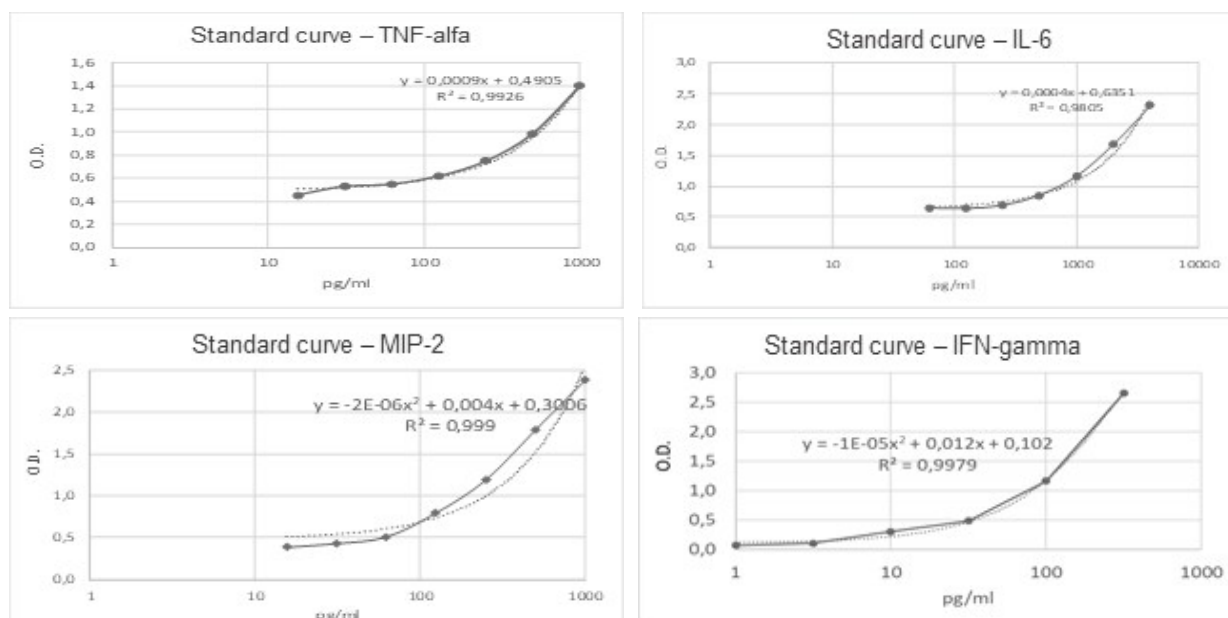


Fig. S5. Representative ELISA standard curves for each factor: TNF-α, IL-6, MIP-2 and IFN-γ.

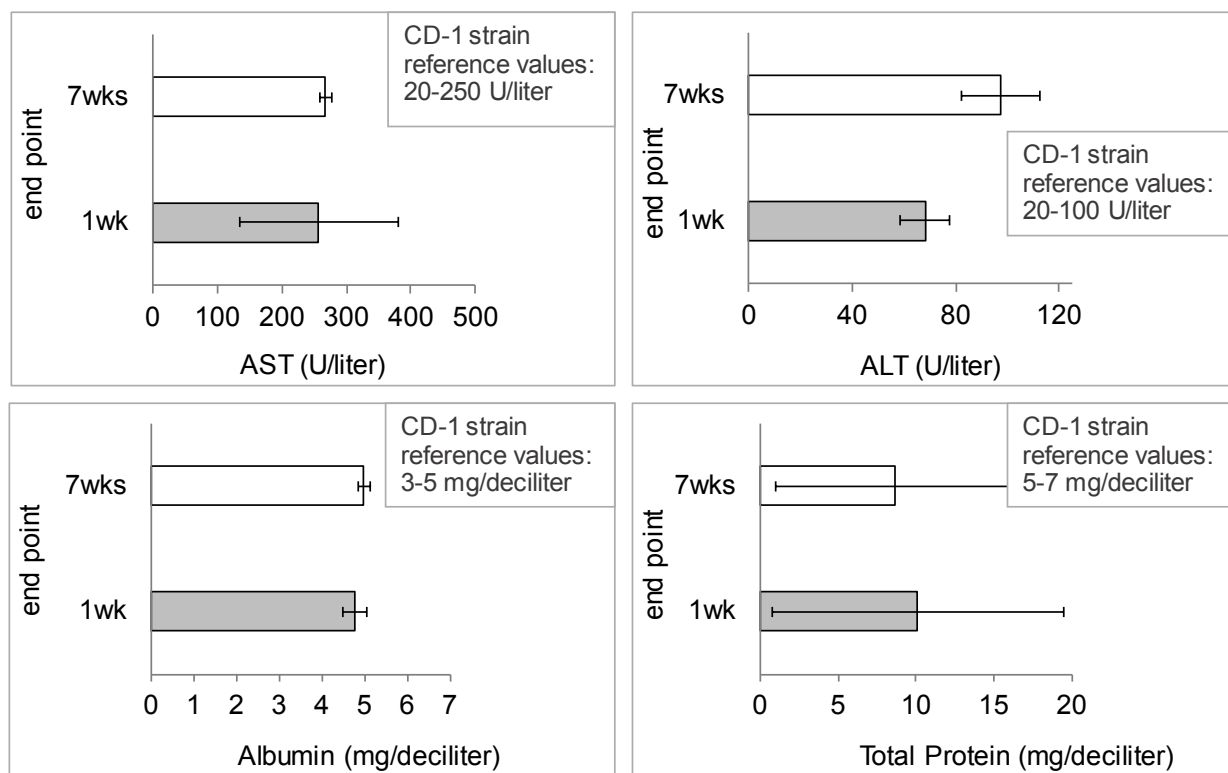


Fig. S6. Biochemical analysis results of AST, ALT, albumin and total protein in mouse blood serum, from animal treated with 10mg/kg of sub-5 SIO-FI nanoparticles, collected at 1week and 7 weeks after i.v. injection. Results represent the mean and standard deviation.