COMMUNICATION

Syntheses of cross-linked polymeric superparamagnetic beads with tunable properties

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Electronic Supplementary Information (ESI)

Materials

All the chemicals not mentioned were analytical reagent grade obtained from Sigma-Aldrich Chemie GmbH, Buchs, Switzerland and were used without further purification. Ultrapure deionized water (Seralpur Delta UV/UF setting, 0.055 μS/cm) was used in all the synthesis and analysis steps. PVA-OH, Mowiol 3-85 was supplied courtesy of Kuraray Specialties Europe GmnH, Germany. Amino-PVA (A-PVA):M12 was supplied courtesy of ERKOL S.A, Tarragona Spain (now Sekisui Specialty Chemicals Europe). MWCO 12-14 kDa cellulose membrane dialysis tubing was used for the dialysis. Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1.5% penicillin streptomycin, 1% amphotericin B and Prolong Gold were all provided by Invitrogen AG, Basel, Switzerland. Phosphate buffered saline (PBS) was used to wash the cells. Paraformaldehyde (PAF) and DAPI were purchased from Sigma-Aldrich Chemie GmbH, Buchs, Switzerland. MTS tetrazolium salt was provided by Promega, Dübendorf, Switzerland.

Chemical characterizations

To titrate the amounts of iron and silicon on the PVA-SPION@silica particles for induced coupled plasma atomic emission spectroscopy (ICP-OES: ICPE-9000 Shimadzu), 80 μL of the particles were dissolved in 920 μL of either 6 M HCl or 2 M NaOH for, Fe or Si titrations, respectively. Then, 5 points standard BET (Micromeritics Gemini 2375) specific surface areas (SSA) were measured on PVA-SPION@silica particles after degassing at 130°C. The BET diameter (dBET) was obtained from SSA measurements using the equation SSA=6000/(ρ*dBET), where ρ is the density of the PVA-SPION@silica particles calculated from the Fe and Si concentrations measured using ICP. The amount of iron was also titrated using magnetic susceptibility (Bartington MS3 magneto-susceptometer)[1].

To determine the remaining amount of PVA in the silica beads, freeze dried particles were thermogravimetrically analyzed (Mettler Toledo TGA/SDTA 851e) from 30 to 800°C (10°C/min) under air (30 mL/min). The loss of mass from 150 to 600°C was assumed to be due to the loss of mass of the PVA.

Calculation of the average density of PVA-SPION silica beads

The density of porous silica (d_{SiO2}) was estimated from the literature[2] to be 1.3 g.cm^{-3}. The density of SPION (d_{Fe2O3}) was taken to be 4.8 g.cm^{-3}. The atomic concentrations of silicon (c_{Si}) and iron (c_{Fe}) were measured using ICP. Then, assuming Si would produce SiO_2, the concentration of silica (c_{SiO2}) was estimated using the formula: $c_{SiO2}=c_{Si}*M_{SiO2}/M_{Si}=c_{Si}*60/28$. Assuming Fe would yield Fe_2O_3, the concentration of Fe_2O_3 (c_{Fe2O3}) was estimated from the formula: $c_{Fe2O3}=c_{Fe}*M_{Fe2O3}/(2*M_{Fe})=c_{Fe2O3}*159.7/111.7$. Then, the mass ratio of Fe_2O_3 ($\%_{Fe2O3}$) was calculated according the equation: $\%_{Fe2O3} = c_{Fe2O3}/(c_{Fe2O3}+c_{SiO2})$. Then, the global density of PVA-SPION@silica (d_{NP}) was calculated:

$$d_{NP} = \frac{c_{Fe2O3} + c_{SiO2}}{\dfrac{c_{Fe2O3}}{d_{Fe2O3}} + \dfrac{c_{SiO2}}{d_{SiO2}}}$$

Calculation of number of SPION per silica bead

These calculations were based first on the TEM sizes of naked SPION (θ_{SPION}) and silica (θ_{Silica}). The density of porous silica (d_{SiO2}) was estimated from literature values[2] to be 1.3 g.cm^{-3}. The density of SPION (d_{Fe2O3}) set at 4.8 g.cm^{-3}. Then, atomic concentrations of silicon (c_{Si}) and iron (c_{Fe}) were measured using ICP. The volume of 1 SPION (V_{SPION}) was calculated assuming a spherical shape: $V_{SPION} = 3/4*\pi*(\theta_{SPION}/2)^3$.

The number of SPION / mL (N_{SPION}) was calculated using the equation:

$$N_{SPION} = \frac{c_{Fe2O3}}{V_{SPION} * d_{Fe2O3}}$$

The volume of SiO_2 (V_{SiO2}) was calculated: $V_{SiO2} = c_{SiO2} / d_{SiO2}$.

The volume of the silica beads (V_{beads}) was calculated: $V_{beads} = 3/4* \pi *(\theta_{silica}/2)^3$.

The number of SPION/bead ($N_{SPION/bead}$) was then calculated using the following equation:

$$N_{SPION/bead} = \frac{N_{SPION}*V_{SPION}+V_{SiO2}}{V_{beads}}$$

Estimation of the final PVA/Fe mass ratio

3 mL of the silica suspensions were freeze-dried and weighed (m_{total} in mg). A few mg of these powders was analyzed by TGA. The loss of mass from 150 to 600°C (above this temperature, the loss of mass was null) was used for the calculation ($\%_{TGA}$) of PVA loss. The concentration of Fe/mL was obtained by ICP measurements (c_{Fe}). The final PVA/Fe ratio was calculated using the following equation:

$$PVA/Fe = \frac{\%_{TGA} * m_{total} \Big/ 3mL}{c_{Fe}}$$

Magnetic susceptibility measurements

The mass specific susceptibility (κ in m^3 kg$_{Fe2O3}$$^{-1}$) was obtained by measuring a volume magnetic susceptibility (χ in SI) on a Bartington MS3 magneto-susceptometer at 300Ka with a MS2G mono frequency sensor for around 1 mL cells operated at (1.3 kHz). Then κ was obtained by dividing χ by the concentrations of SPION (obtained as explained before) in kg$_{Fe2O3}$ m^{-3}.

Magnetic Resonance Imaging

T_2 signal loss and quantifiable dUTE MRI images were acquired in a phantom of varying concentrations up to 200μg/ml made with deionised water in 2ml tubes, placed in a container of water to avoid susceptibility artifacts. MR employed a Siemens Magnetom® Trio 3T clinical scanner

(Erlangen, Germany) using the standard 4cm loop coil. Sequence parameters were as follows: T₂ STIR conventional 'signal loss' SPION imaging: 2D, TR/TE 3700/20ms, Flip angle 150°, Resolution 0.156 mm, FOV 60mm, Slice thickness 1mm. dUTE: 3D isotropic resolution 448 and 80 mm FOV, Resolution 180µm in all three dimensions, 50000 radial projections, TE(1)/TE(2) 0.07ms/2.46ms (for in-phase fat/water image), TR 9.6ms (in vivo 100 segments), Flip angle 10°. Difference Ultra-short Echo time imaging (dUTE) was used for iron oxide particles positive contrast detection and quantification. SPION detection by dUTE sequence consisted of the acquisition and subtraction of two echo times (ultrashort TE, and short TE(2)) leading to positive contrast from short T_2^* species and reduced signal elsewhere.

Biological characterizations

RAW 264.7 (mouse leukemic monocyte macrophage cell line) and HeLa cells were used in this work and were incubated at 37°C in a 5% CO_2 atmosphere.

To evaluate the 24 hours cytotoxicity of the magnetic silica particles, the MTS assay was selected. RAW 264.7 cells were plated at 85000 cells/well and HeLa cells at 20000 cells/well in a 96-well plate at 37°C in a 5% CO_2 atmosphere. For each condition, wells with no cells inside were prepared to determine if the nanoparticles affected the test. After 24 h of incubation, the medium was replaced by 100 µL of the various nanoparticles synthesized and diluted in medium at various nanoparticle concentrations (0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL). For each condition, triplicates were performed. After 24 h of incubation, the medium was removed, and 100 µL of MTS reagent, previously diluted 6 times, was added. After two hours of incubation, the absorbance at 490 nm was recorded using an Infinite M200 plate reader from TECAN. The cell viability (%) was calculated relatively to the control wells, where only medium was added to the cells (Figure E1 for RAW cells and Figure E2 for HeLa cells).

For internalization of confocal observations, 200000 RAW macrophages and 85000 HeLa cells were cultivated in 6-well plates at 37°C and 5% CO_2. After removing the old medium, 1 mL of medium with 0.4 mg/mL of the different particles added for an incubation of 24 h at 37°C, 5% of CO_2. The medium was then removed, and the cells were washed three times with PBS. Then, 1 mL of fresh medium with 10 µL of DAPI solution (100 µg/mL) was added and incubated with the cells for 10 minutes at 37°C, 5% of CO_2. After incubation, the medium was removed, and the cells were washed three times with PBS. Next, 5 mL of 4% paraformaldehyde (PAF) was added and incubated with the cells for 15 minutes at 37°C, 5% of CO_2. After removal of the PAF, the cells were washed three times with PBS. The cover slips were fixed on microscopy slides using Prolong Gold. The polymerization was allowed to occur at room temperature for 24 h, protected from the light. Nail polish was used to seal the cover slips on the microscopy slides before the slides were examined on an inverted confocal microscope (Carl Zeiss LSM 700 Invert, software: Zen 2009) with a 405 nm laser for DAPI detection (550 nm beam). The confocal imaging of HeLa cells are given in Figure E3.

Table E1: Characterizations of PVA-SPION with PVA/Fe ratio from 0 to 9 and with or without fluorophore

Initial PVA/Fe ratio	FITC (%)	TEM size (nm)	PCS size (nm)	Zeta Potential (mV)
R_i0	No	7.5 ± 2.0	14 ± 2	+26 ± 2
R_i1	No	7.5 ± 2.0	34 ± 3	+9 ± 5
R_i2	No	7.5 ± 2.0	34 ± 3	+18 ± 5
R_i4	No	7.5 ± 2.0	27 ± 3	+16 ± 4
R_i5	No	7.5 ± 2.0	45 ± 3	+25 ± 2
R_i7	No	7.5 ± 2.0	31 ± 3	+21 ± 1
R_i9	No	7.5 ± 2.0	30 ± 3	+20 ± 2
R_i9^*	Yes (10%)	7.5 ± 2.0	25 ± 3	+26 ± 2

FITC (%): mass percentage of A-PVA-FITC added compared to the A-PVA mass already added; TEM size: diameter measured by the counting diameters on TEM pictures; PCS size: hydrodynamic number weighted diameter
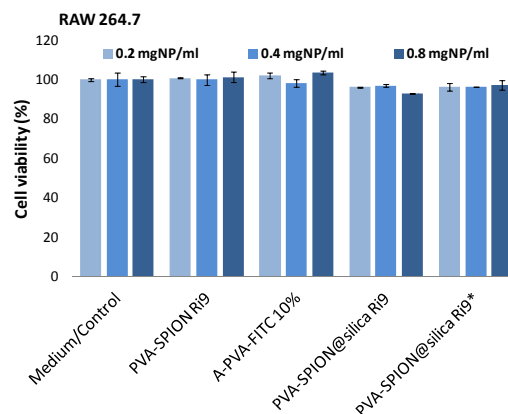

Figure E1: Cytotoxicity tests comparing to the percentage of viability of RAW 264.7 cells grown in medium and cells incubated with nanoparticles after 24 hours. From left to right: PVA functionalized SPION with a PVA/Fe mass ratio R_i9; Mixture of PVA-OH, Amino PVA and fluorescein functionalized Amino-PVA in the same proportion and quantity than PVA functionalized SPION; silica beads encapsulated PVA-SPION R_i9 and silica beads encapsulated PVA-SPION R_i9^*. Concentrations used: 0.2, 0.4 and 0.8 mg$_{NP}$/mL.
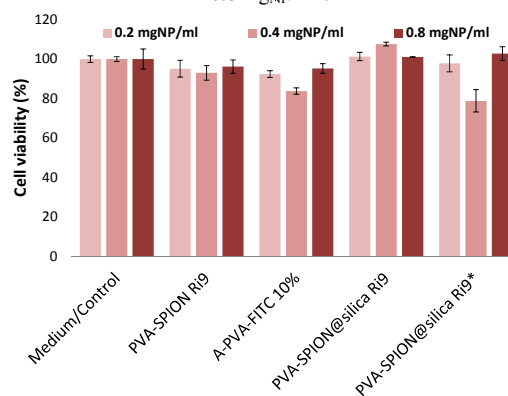

Figure E2: Cytotoxicity tests comparing to the percentage of viability of HeLa cells grown in medium and cells incubated with nanoparticles after 24 hours. From left to right: PVA functionalized SPION with a PVA/Fe mass ratio R_i9; Mixture of PVA-OH, Amino PVA and fluorescein functionalized Amino-PVA in the same proportion and quantity than PVA functionalized SPION; silica beads encapsulated PVA-SPION R_i9 and silica beads encapsulated PVA-SPION R_i9^*. Concentrations used: 0.2, 0.4 and 0.8 mg$_{NP}$/mL.
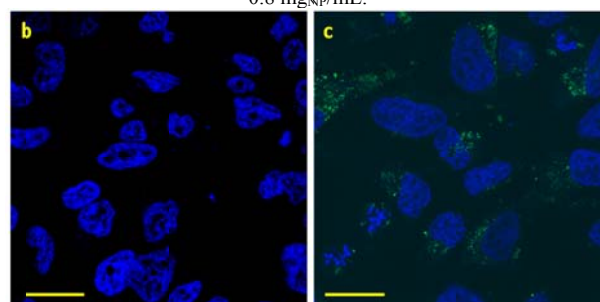

Figure E3: b and c: Confocal imaging of RAW 264.7 cells incubated with: b) 0.4 mg PVA-SPION@silica Ri9 per mL and c) 0.4 mg PVA-SPION@silica Ri9* per mL. Blue colour: nuclei labelled with DAPI. Green colour: FITC labelled nanoparticles. Scale-bar: 20 µm

1. L. Maurizi, U. Sakulkhu, A. Gramoun, J.-P. Vallee, and H. Hofmann, *Analyst*, 2013.
2. M. I. Fridland, *Chem. Pet. Eng.*, 1968, **4**, 126–129.