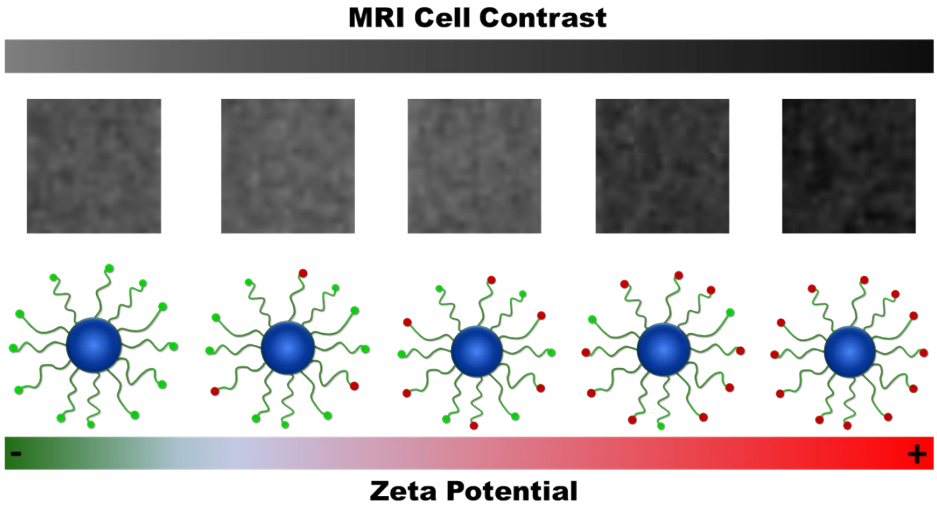
Tailoring the surface charge of dextran-based polymer coated SPIONs for modulated stem cell uptake and MRI contrast.

Supporting information

Graphical abstract



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Supplementary Information

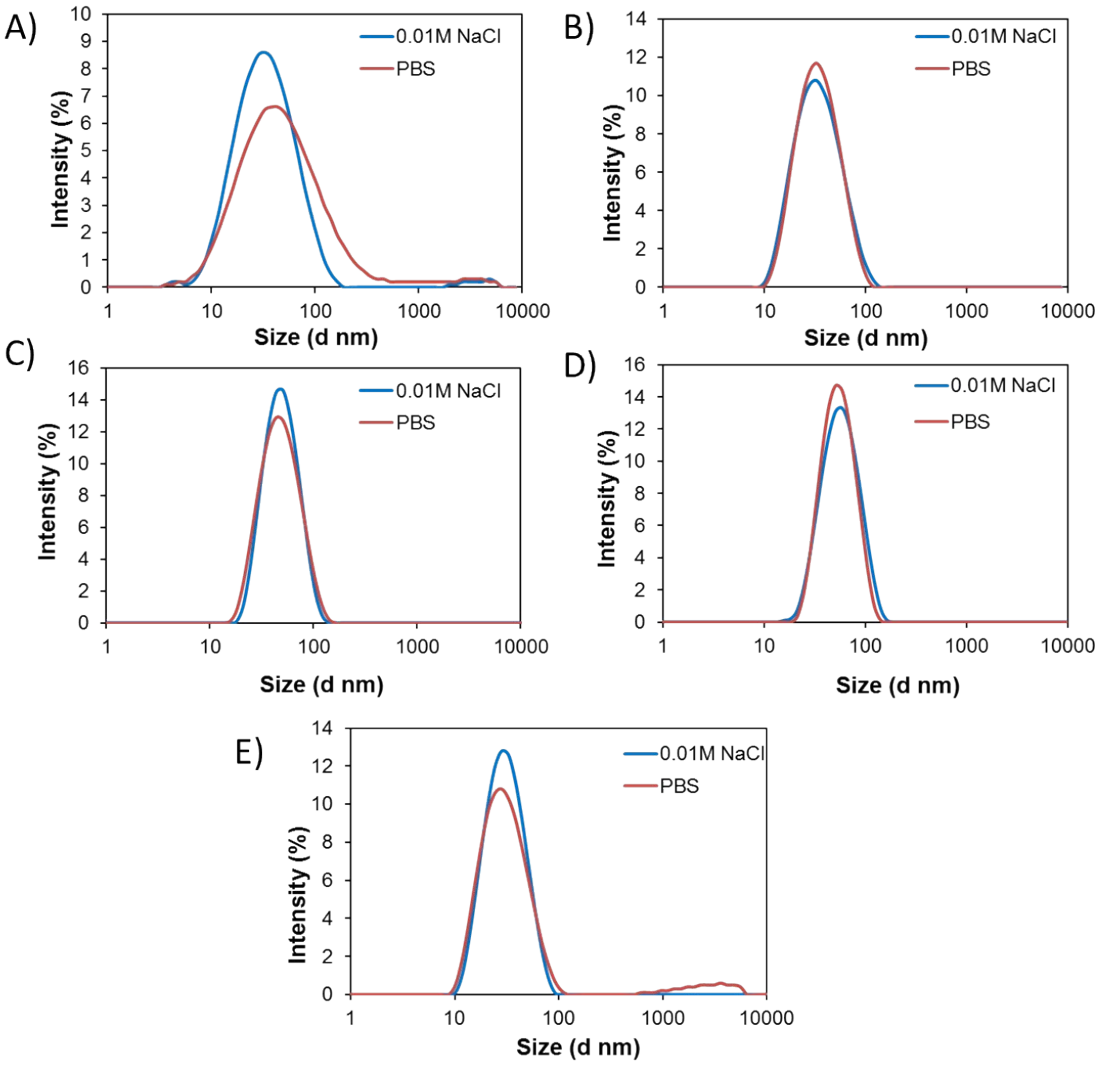


Figure S1 Particle size distribution in 0.01M NaCl and PBS of SPIONs containing A) 0% B) 25% C) 50% D) 75% E) 100% DEAE-dextran (weighted by intensity) as measured by DLS.

Table S1 DLS and zeta potential values in 0.01M NaCl

Sample	Z-Avg (nm)	PDI	Zeta Potential (mV)	Standard Deviation
0% DEAE-Dex	28.11	0.269	- 1.54	± 9.62
25% DEAE-Dex	29.83	0.197	+ 8.09	± 15.1
50% DEAE-Dex	44.81	0.121	+ 9.12	± 21.1
75% DEAE-Dex	49.79	0.165	+ 9.66	± 19.3
100% DEAE- Dex	26.95	0.163	+ 18.2	± 29.7

Table S2 DLS values in PBS

Sample	Z-Avg (nm)	PDI
0% DEAE-Dex	34.94	0.418
25% DEAE-Dex	29.64	0.188
50% DEAE-Dex	42.84	0.154
75% DEAE-Dex	47.84	0.153
100% DEAE- Dex	28.25	0.248

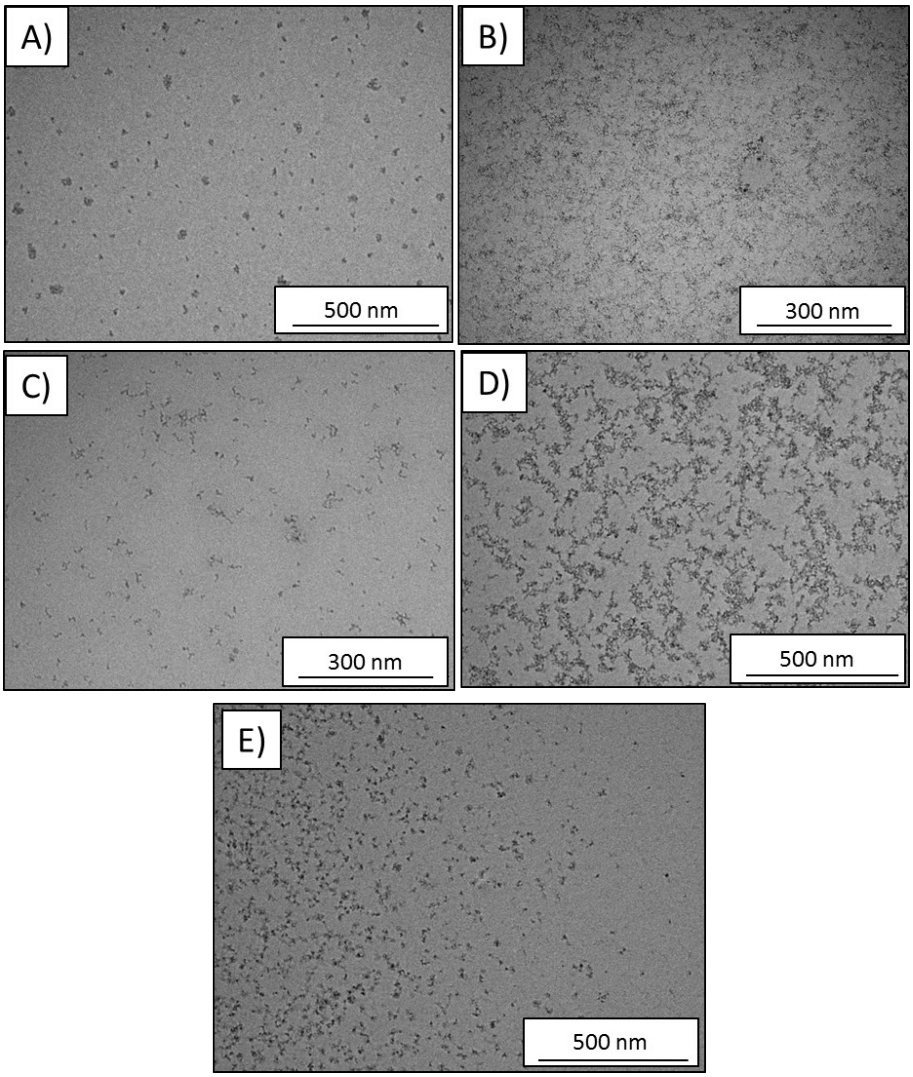


Figure S2 TEM images of SPIONs produced using (A) 0% (B) 25% (C) 50% (D) 75% (E) 100% DEAE-dextran. Images were collected using a FEI 120 kV Tecnai G2 Spirit BioTWIN TEM at an accelerating voltage of 120 kV. Samples were prepared on Agar Scientific 200 mesh copper grids coated in a Formvar coating.

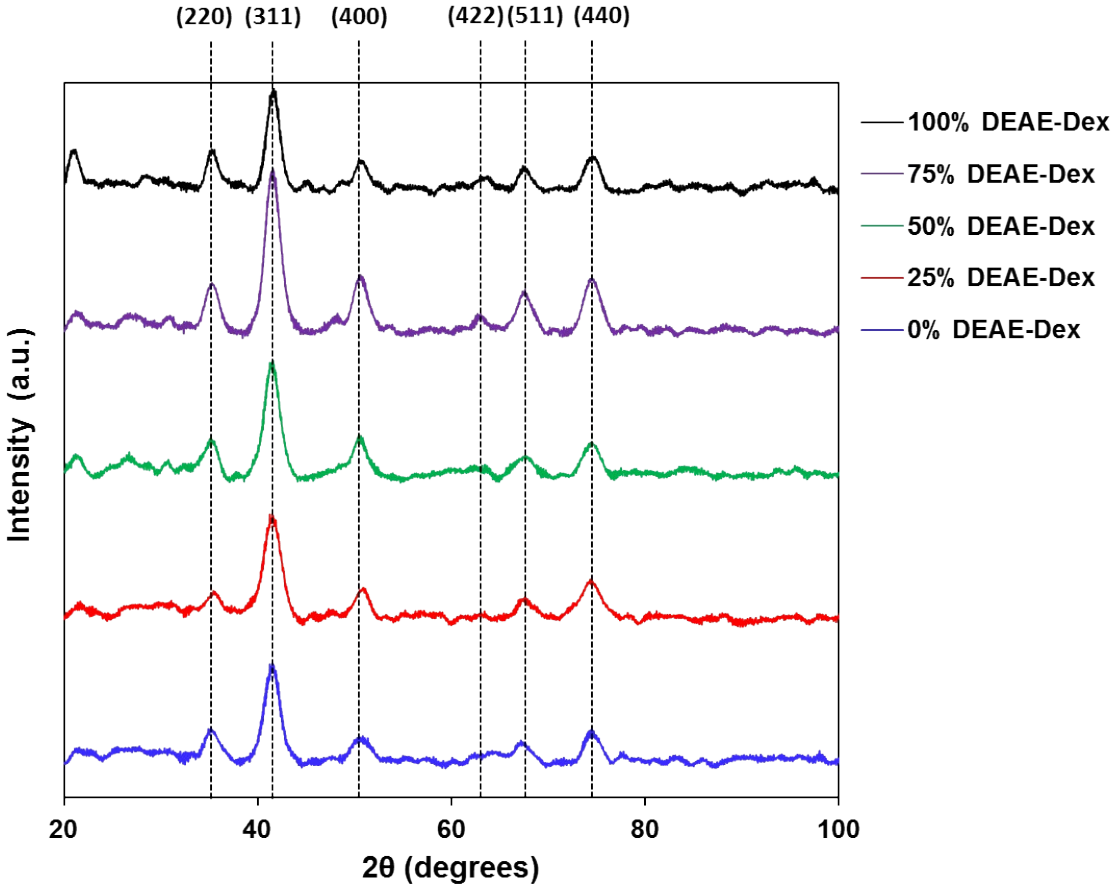


Figure S3 pXRD patterns for SPIONs produced with varying amounts of DEAE-dextran as measured using CoK_{α} radiation (after background subtraction).

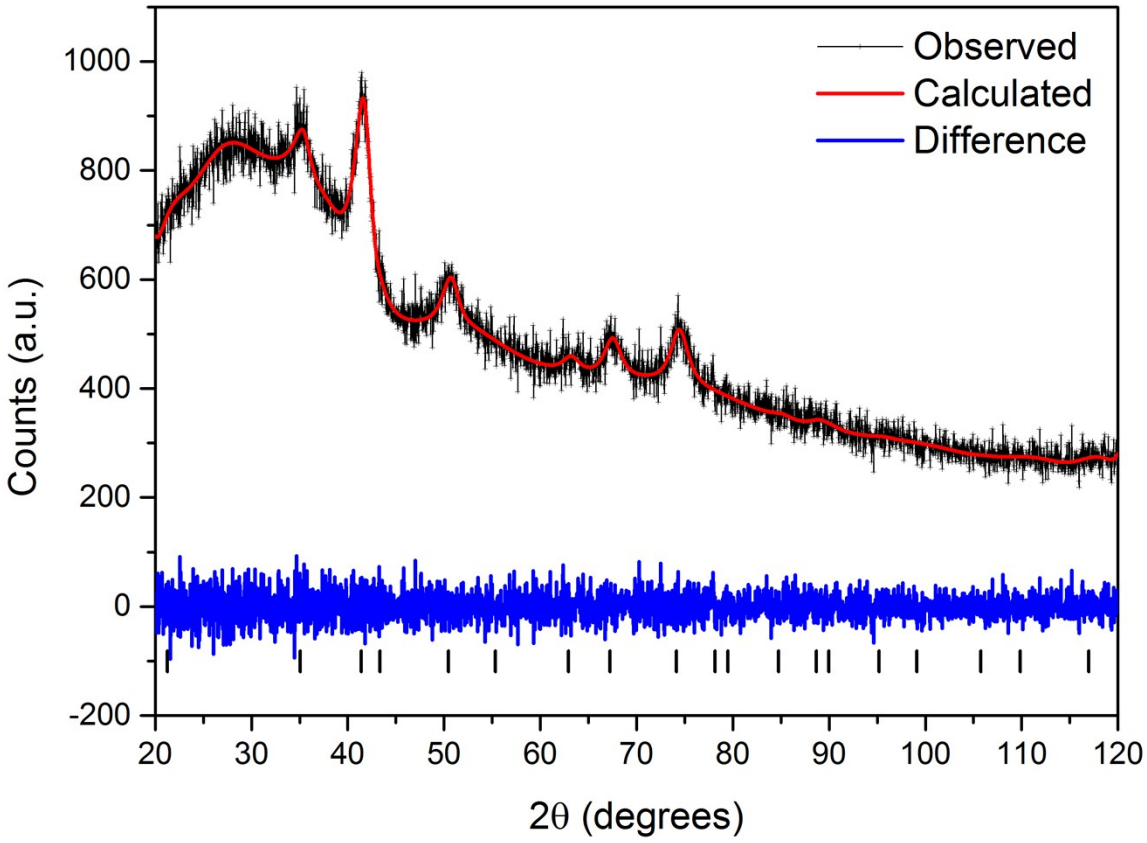


Figure S4 Pawley refinement of pXRD pattern of sample synthesised with 0 % DEAE-dextran.

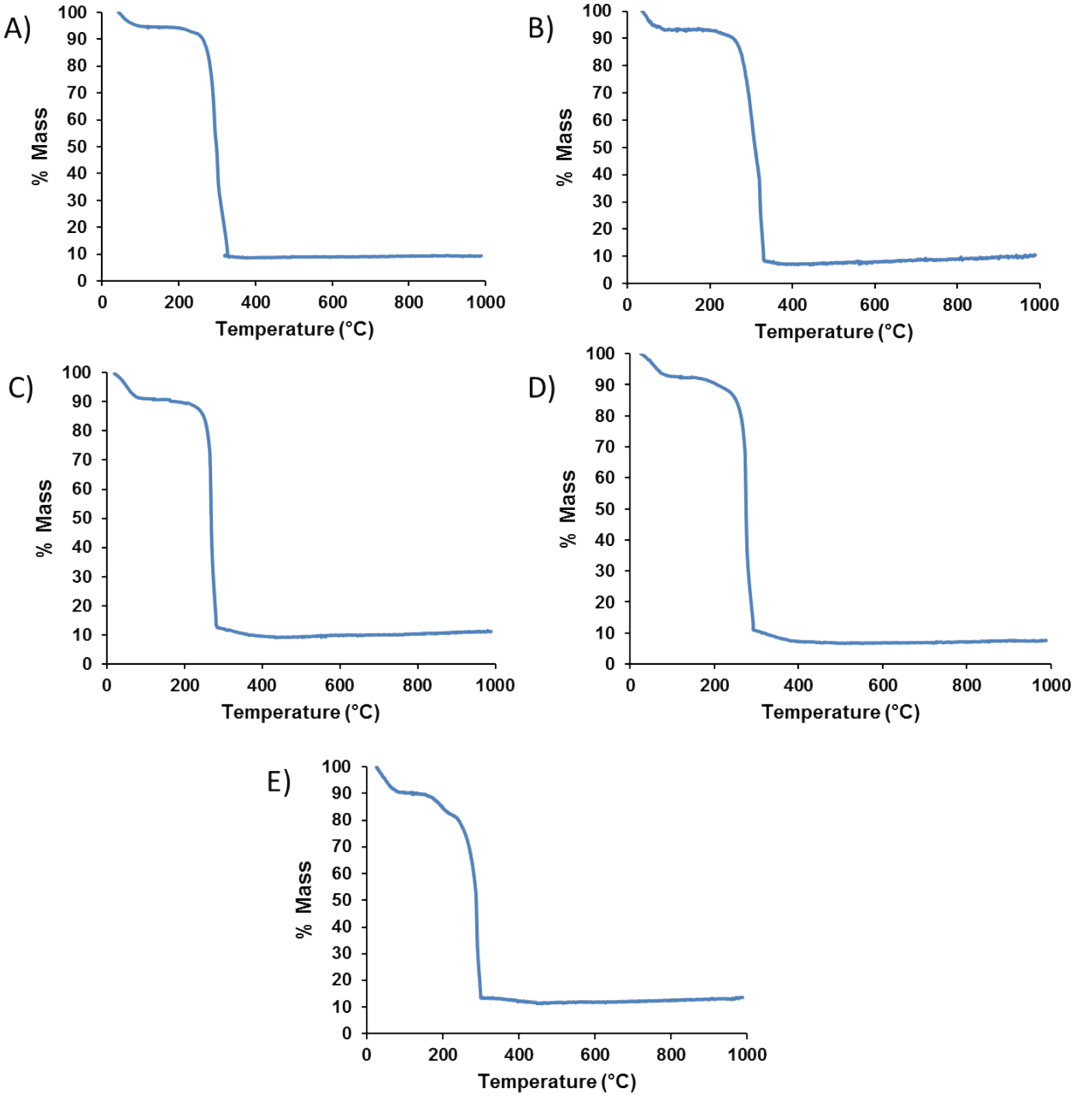


Figure S5 Thermogravimetric analysis (TGA) weight loss curves for SPIONs produced using (A) 0% (B) 25% (C) 50% (D) 75% (E) 100% DEAE-dextran

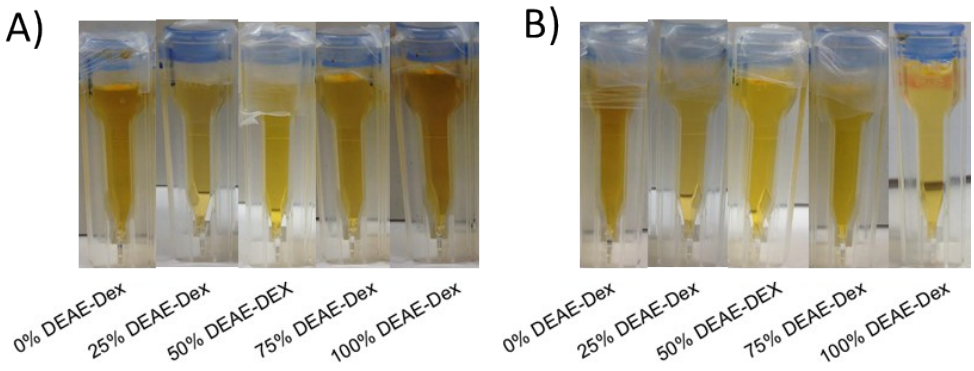


Figure S6 Images of samples (50 µg/ml [Fe]) at t=14 days which were incubated at 37°C and normalised to t=0 in (A) PBS and (B) DMEM+FCS.

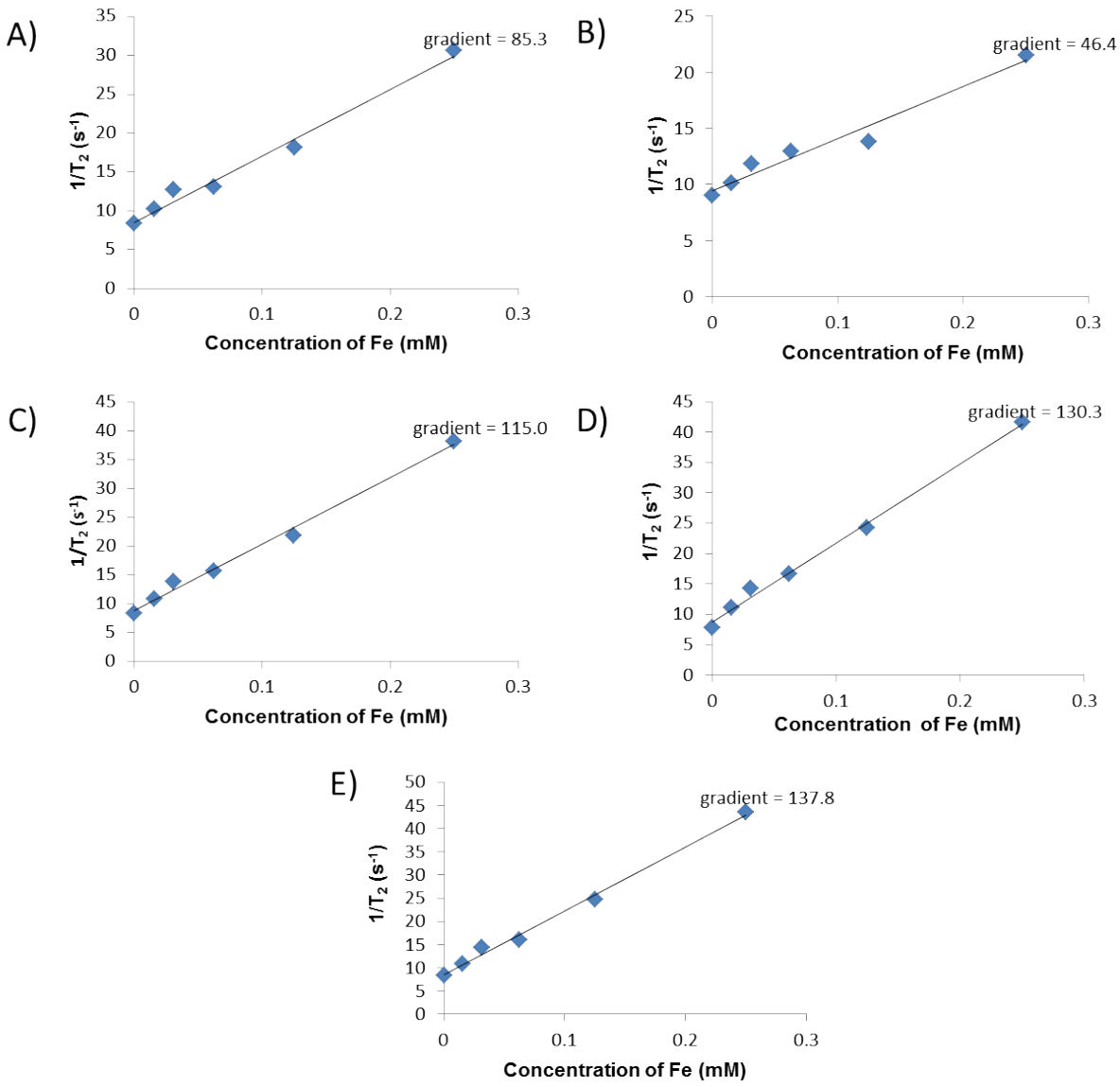


Figure S7 Relaxivity plots obtained at 7 T for SPIONs produced using (A) 0% (B) 25% (C) 50% (D) 75% (E) 100% DEAE-dextran.

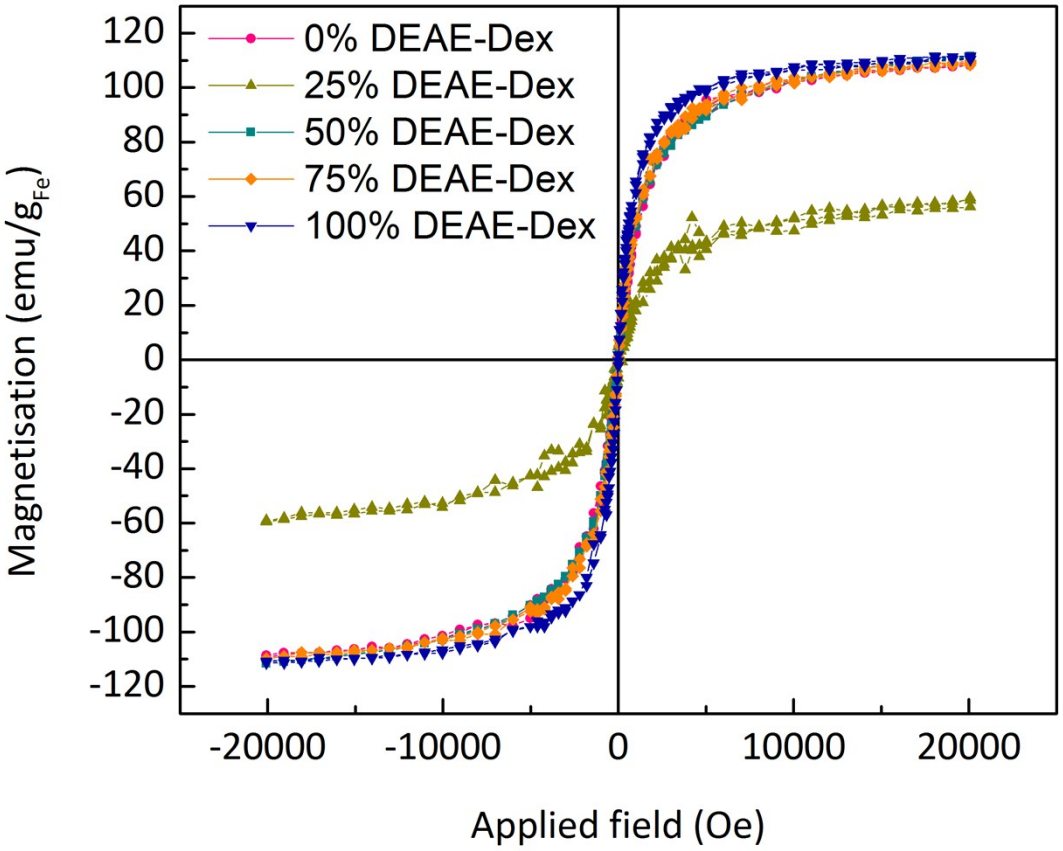


Figure S8 Magnetisation curves measured at 300K on freeze dried SPIONs synthesised with varying ratios of DEAE-dextran.

Table S1 Magnetic relaxation and magnetisation values of SPIONs samples

Sample	Relaxivity ($mM^{-1}s^{-1}$)	R^2	Pearson's R	Magnetisation at 2T (emu/g Fe)
0% DEAE-Dex	85.3	0.99	0.99	108
25% DEAE-Dex	46.4	0.96	0.98	57
50% DEAE-Dex	115.0	0.99	0.99	110
75% DEAE-Dex	130.3	0.99	0.99	110
100% DEAE-Dex	137.8	0.99	0.99	111

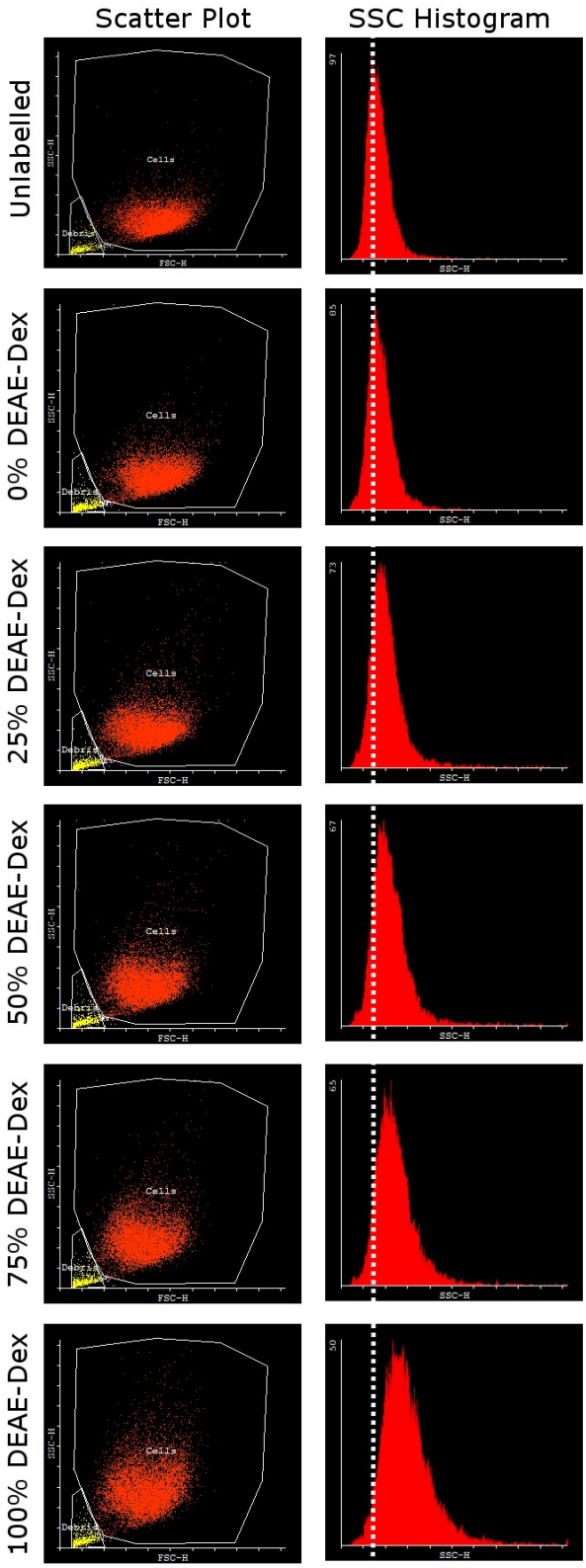
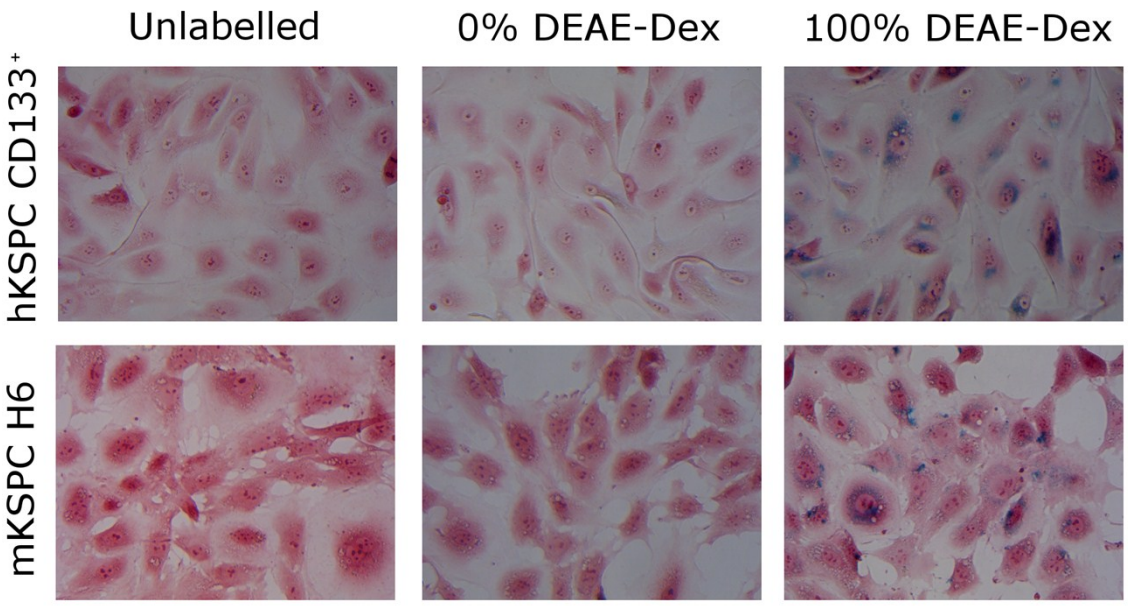


Figure S9 Scatter plots (forward vs side scattered light) of cells labelled for 24 h with 100 µg/ml [Fe] of each SPION. Gates were set for cells (red) and debris (yellow). The right panels display the histogram of the side scatter intensity of the events in the "cell" gate. The dashed lined represents the position of the peak obtained for controls cells and is displayed in all plots as a reference to highlight the shift of the population, which corresponds to the uptake of SPIONs.



Cell Line	Mean Magnetic Velocity (μm/s)	
	0% DEAE-Dex	100% DEAE-Dex
hKSC CD133$^+$	19 ± 12	124 ± 80
mKSC H6	28 ± 14	117 ± 40

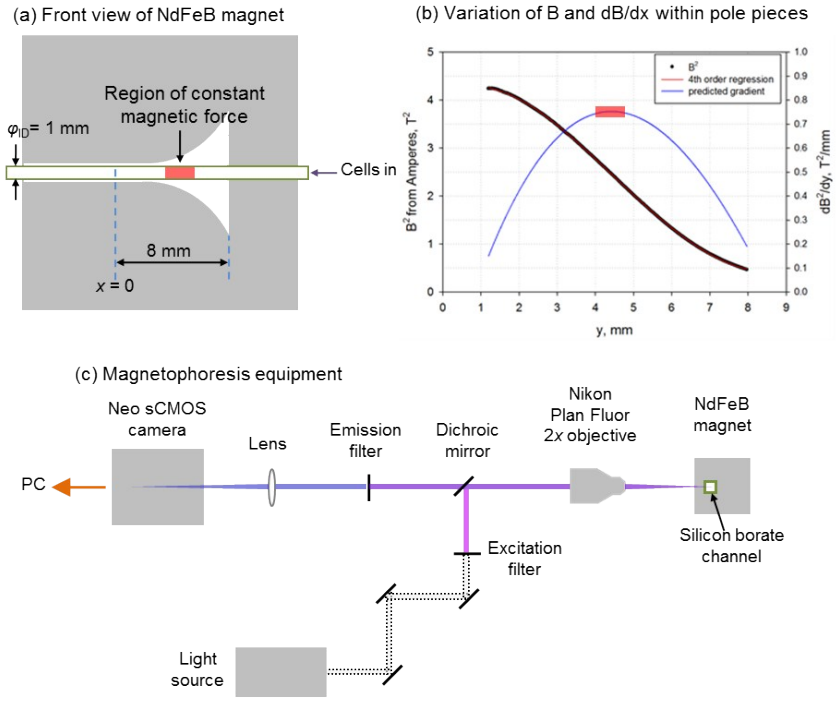
Figure S10 Prussian blue staining (counterstained with pararosaniline) of cells labelled with SPIONs synthesized with 0% or 100% DEAE-Dex (50 μg/ml [Fe] for 24h). Top panel corresponds to cells isolated from human infant kidneys and sorted for CD133 (a stem/progenitor cell marker as described in E. Ronconi, et al. J Am Soc Nephrol., 2009, 20(2), 322-332) and the bottom panel to a mouse kidney-derived cell line (described in C. F. Mora, et al, Stem Cells Dev., 2012, 21, 296–307) . The table summarizes the mean magnetic velocities as measured via cell tracking velocimetry, where the error corresponds to the standard deviation of the population.

Cell Tracking Velocimetry

The microscope consists of a modified Nikon AZ100 body with an AZ-FL epi-fluoresence attachment (Nikon Instruments, Surrey, UK). Magnetically loaded cells are stained with DAPI (Sigma-Aldrich, 32670-5MG-F) and suspended in a 30 % glycerol (v/v) and injected into a square silicon borate capillary tube (Viracon, Owatanna, MN, USA; inner diameter 1 mm, 0.2 mm wall) and placed into a NdFeB magnet (provided by Lee

Moore) with specially shaped pole pieces that produces a magnetic field, which varies from 0.71 T to 2.05 T; this magnetic field gradient has a constant value of 300 mT/mm for approximately 1 mm.

Cells were visualised using fluorescence. Fluorescence imaging was performed using an Intensilight C-HGFI pre-centred mercury-fiber illuminator, which passes through an excitation filter (λexcitation = 358 nm, BP 50 nm) and focussed onto the sample through a Nikon Plan Fluor 2x objective (45 mm working distance, NA = 0.4). Emitted light is collected through the objective, passes through a dichroic mirror and through an emission filter (λemission = 461 nm, BP 50 nm). Light is focused using a 0.6x reducing lens and 2.5x c-mount onto an Andor Neo sCMOS camera with an effective pixel size of 2.145 µm at a readout rate of 200 MHz. Kinetic series of magnetically loaded fluorescently stained cells were acquired and saved as a .sif file using the Andor Solis acquisition software. The kinetic series for all CTV measurements consisted of 400 frames with a frame rate of 8.74 fps.



Scheme S1 (a) Front view of the NdFeB magnet and channel in which cells are suspended for magnetic characterisation (b) variation of magnetic field and field gradient within the pole pieces and indicating the magnetic pole pieces that give rise to the spatially varying magnetic field gradient and (c) simplified schematic of the magnetophoresis equipment used to measure magnetic velocity of cells.

Magnetic velocity quantification was performed using MacBiophotonics ImageJ (*https://www.macbiophotonics.ca*). Each kinetic series was imported as a stack of 8-bit tif images and then cropped to include only the cells within the area of constant magnetic field gradient (~ 1 mm in width). Areas close to the top and bottom of the channel were also cropped so as to remove as the effects of sticking to the inner surface of the glass channel which were observed to affect propagation. The frames were combined to form a stack and then subsequently averaged using the 'Z-project average intensity' function. This value was subtracted from the entire stack to both improve the detection of labelled cells but also remove those that become stuck to the capillary. A threshold was then applied to convert the resulting stack to binary. Spurious pixels were removed using the erode function, after which the dilate function was applied to enhance the size of the cell nuclei for tracking. Finally, the stack was inverted and single cells were tracked between successive frames using the MTrack2 plugin (h*ttp://valelab.ucsf.edu/~nstuurman/ijplugins/MTrack2.html*). The minimum size of object tracked was chosen to ensure the maximum numbers of cells were tracked, but so as to exclude contributions from spurious pixels and out-of-focus cells. Similarly, the maximum velocity and minimum number of frames were chosen to track the maximum number of cells but to reduce label interchange that can occur during tracking of trajectories when adjacent cells become to close. The tracks of all cells are post-processed to remove any static cells that are adhered to the inner edges of the channel and to check there is no interchange of label between individual cells.