Supporting Information

Magnetic Conjugated Polymer Nanoparticles Doped with a Europium Complex for Biomedical Imaging

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Experimental section

Materials and methods

Poly(9-vinylcarbazole) (PVK, MW 50,000), tris(dibenzoylmethane)mono(1,10phenanthroline)europium(lll) ($Eu(dbm)_3phen$), poly(ethyleneglycol) methyl ether-block-poly(lactide-co-glycolide) copolymers (PEG-PLGA) with 50:50 ratio of lactide/glycolide and differing molecular weights, $PEG_{2\ kDa}$-$PLGA_{4\ kDa}$ (6 kDa); $PEG_{2\ kDa}$-$PLGA_{15\ kDa}$ (17 kDa); $PEG_{5\ kDa}$-$PLGA_{55\ kDa}$ (60kDa, tetrahydrofuran (THF; ReagentPlus®, ≥99.9%) were purchased from Sigma Aldrich and used as received. Oleic acid-capped iron oxide nanoparticles, 6.5 nm, in heptane (BioChemika 07318) or magnetic iron oxide nanoparticles (10-40 nm, 0.8-1.4% total solid in heptane) were purchased from Sigma-Aldrich (England, UK) and used as received. Ultrapure water was used throughout (18.2 MΩ).

Preparation of europium-doped PVK polymer nanoparticles

A factorial approach was used to determine the optimal component ratios to produce self-assembling PLGA-PEG nanoparticles containing PVK, the europium complex, and iron oxide nanoparticles. Two factors were investigated: the PLGA-PEG structure (i.e. molecular weight; 6, 17 and 60 kDa) and the mass percentage of PLGA-PEG in each system (63% and 89%). For all systems, the ratio of the Eu-complex to PVK content remained constant.

In a typical example, PVK (1 mg) and $Eu(dbm)_3phen$ (0.2 mg) were dissolved in 10 mL THF. Subsequently, either 3.2 or 10.9 mg of the PLGA-PEG copolymer were dissolved in the stock solution at room temperature, followed by the addition of 0.02 mL of SPIONs (0.8-1.4% (w/w%) of total solids) to the polymer/THF solvent phase. The mixture (2 mL) was added dropwise to 5 mL ultrapure water under ultrasonication at room temperature (~35 kHz) for 20 minutes. The nanoparticle dispersions were then stored inside a fume hood to allow complete evaporation of THF and the volume re-adjusted to 5 mL with water. Suspensions without SPIONs had a final total solids concentration of 177 µg/mL (63% PLGA-PEG) and 484 µg/mL (89% PLGA-PEG). Suspensions with SPIONs have a final total solid concentration of 0.257 µg/mL (63% PLGA-PEG) and 0.564 µg/mL (89% PLGA-PEG).

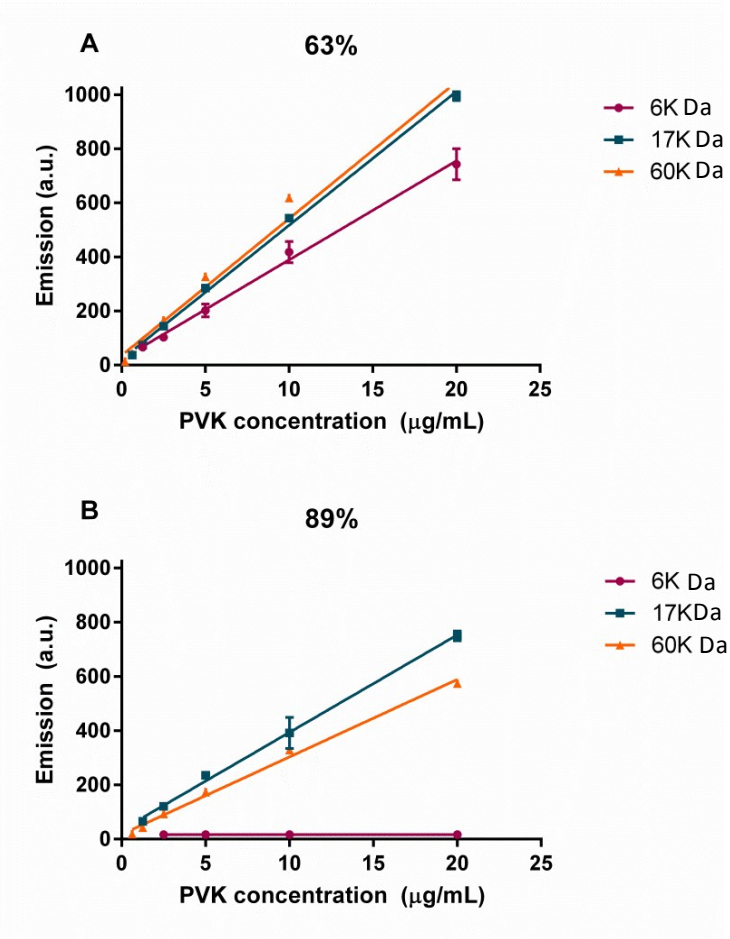
Instrumentation

The size of the particles was assessed by dynamic light scattering (DLS) using a Zeta sizer NanoZS (Malvern Instruments Ltd, UK) at 25 °C. The emission was measured on a LS50B, Perkin Elmer spectrometer with a 365 nm excitation wavelength. Absorption spectroscopy was performed using a Perkin-Elmer Lambda 800 UV/vis spectrometer. Determination of limits of detection (LODs) and limits of quantification (LOQs) for the nanoparticles was monitored to compare the emission outputs of the 6 kDa, 17 kDa, and 60 kDa PLGA-PEG. To monitor the effect of water and cell culture media on the nanoparticle optical properties, the photoluminescence (PL) was measured at 0 and 7 days for optimised systems. Transmission electron microscopy (TEM) was performed on an FEI Tecnai T20 at 200 kV for high resolution imaging. Samples were drop cast and dried on carbon film copper grids. An absolute quantum yield (QY) measurement system (C9920-02G; Hamamatsu) was used to record the QY of samples using an integrating sphere capable of nitrogen gas flow and a CCD spectrometer for detecting the whole spectral range simultaneously. An excitation light source with a wavelength of 365 nm and a bandwidth of 5-7 nm was used. The spectra were averaged from 20 repeat recordings. Magnetic measurements were done at the London Centre of Nanotechnology using a physical property measurement system (PPMS, Quantum design) 3.8 mg of the 63% PLGA-PEG (P6OK) was weighed out and the applied field ranged from -14kOe to 14kOe at 310 K.

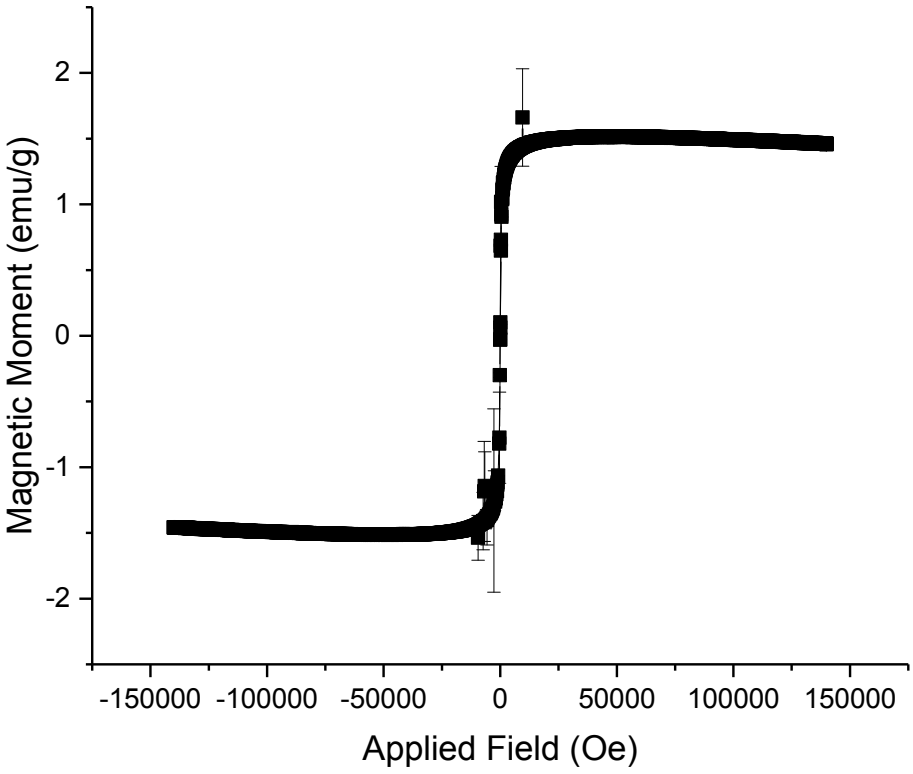
CPN Interactions with primary T Cells

Peripheral blood mononuclear cells (PBMCs) were isolated using Lymphoprep (Stemcell Technologies, CA) and T cells expanded by culturing for 48 h in RPMI media supplemented with 1 µg/mL phytohaemagglutinin (PHA), and subsequently 5 days with 20 ng/mL recombinant human Interleukin-2 (IL-2) at 37°C in a humidified incubator (5% CO_2). PBMCs were transferred to a sterile 8 square well microplate at 3×10^5 cells/well. Optimal conjugated polymer nanoparticles (CPN)

suspensions were studied: (100 µL; equivalent to 12 µg total solids per well) were added to 150 µL cell culture media and incubated for 1 h with cells. Following incubation, cells were gently washed three times with warm phosphate-buffered saline (pH. 7.0) and imaged live every 5 seconds for 5 minutes, using a Nikon A1R+ confocal, through a ×20 objective lens (0.75 N_A), combined with a Nikon Ti-E inverted microscope system. A 488 nm diode pumped solid state (DPSS) laser was used for excitation (~8 mW) and emission was collected in a 570 - 620 nm (visible red) channel.



Supporting figure 1 - CPN emission as a function of PVK concentration (µg/mL). A) 63% PLGA-PEG compositions; B) 89% PLGA-PEG compositions.



Supporting figure 2 - Magnetic measurements from SQUID magnetometer, showing the M-H loops at 310 K of 63% P60K NP with SPIONs.