Supplementary Information

Colloidal Assemblies of Oriented Maghemite Nanocrystals and their NMR Relaxometric Properties

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S1. Toxicity experiments

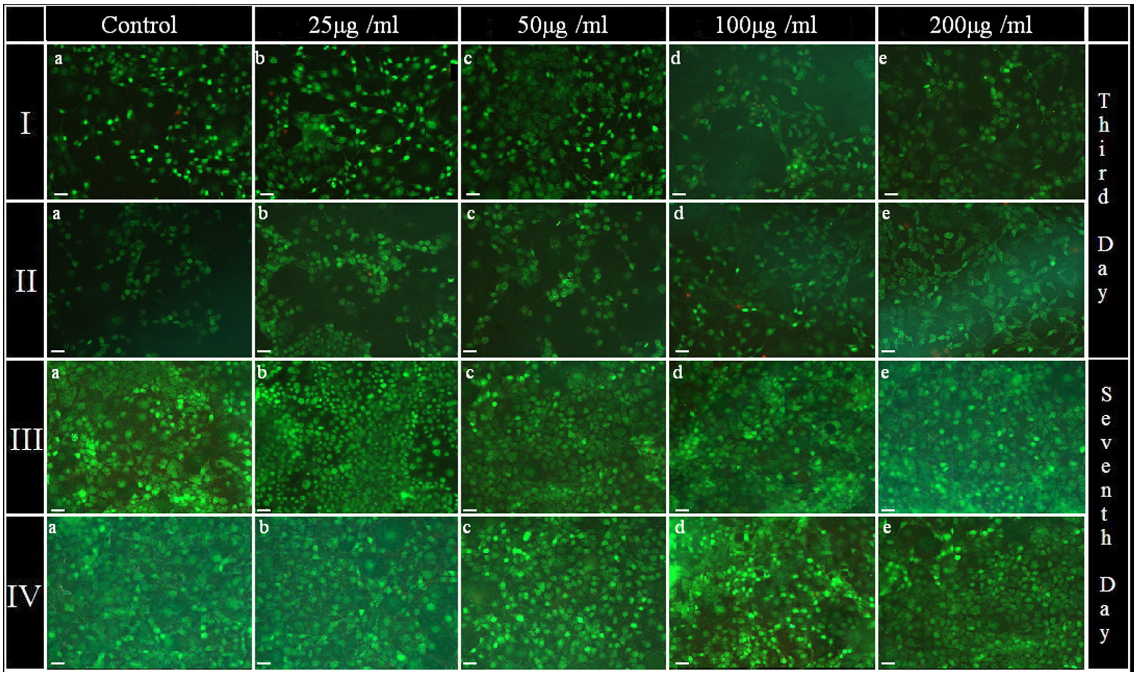

Figure S1. Fluorescence microscopy images of live (green) and dead (orange-red) fibroblast cells cultured without CNCs (a), with 85.6 nm CNCs (I, III), and 50.2 nm CNCs (II, IV) under different concentrations (25μg/mL-b, 50 μg/mL-c, 100 μg/mL-d, 200 μg/mL-e), after 3 (I,II) and 7 (III,IV) days of culture. The scale bar corresponds to 50 μm.

S2. Calculation of the intra-cluster volume fraction of the magnetic material[1]

Table S1. (Copy of Table 1) Parameters utilized for the calculation of the intra-cluster magnetic material volume fraction and the determination of the transverse relaxivity regime where CNCs belong. These entail: D_{hydro}, the hydrodynamic diameter of the CNCs; $f_{m\ \gamma\text{-Fe2O3}}$, the weight fraction of the iron-oxide in a known mass of dried nanocluster powder as derived by the thermogravimetric (TGA) measurements; φ_{intra}, the intra-cluster volume fraction of the magnetic material; M_S, the saturation magnetization of the CNCs at room temperature; M_V, the volume magnetization; $\Delta\omega$, the Larmor frequency shift; τ_D, the translational diffusion time.

Sample	**D_{hydro} (nm)**	**$f_{m\ \gamma\text{-Fe2O3}}$ (%)**	**φ_{intra}**	**M_V (10^5 A/m)**	**$\Delta\omega\ \tau_D$**	**Regime**
Endorem®[1]	80	63.8	0.23	0.77	4.4	SDR
50.2 nm CNCs	78.6	86.8	0.60	1.95	4.9	SDR
85.6 nm CNCs	121.8	92.1	0.72	2.37	18.2	SDR

The transverse relaxivity (r_2) regime in which the CNCs belong is determined mainly by three quantities, which are material-dependent characteristics, namely: the volume magnetization (M_V), the volume fraction of the magnetic material (φ_{intra}) and the hydrodynamic size (D). [1]

The intra-cluster volume fraction (φ_{intra}; Table S1) is calculated by the formula:

$$\frac{\rho_{\gamma-Fe_2O_3}(1-f_{m\,\gamma-Fe_2O_3})}{\rho_{PAA} f_{m\,\gamma-Fe_2O_3}} = \frac{1-\varphi_{intra}}{\varphi_{intra}} \tag{1}$$

where $\rho_{\gamma\text{-Fe2O3}} = 4900$ kg/m^3, $f_{m\ \gamma\text{-Fe2O3}}$ the weight fraction of the iron-oxide in a known mass of dried nanocluster powders was estimated from the thermogravimetric (TGA) measurements and $\rho_{PAA} = 1150$ kg/m^3 the density of the polyacrylic acid.[2]

The normalized volume magnetization M_V*, in A/m, can be calculated by the formula:

$$M_V{}^* = \varphi_{intra} \times M_S \times \rho_{\gamma-Fe_2O_3} \tag{2}$$

where the CNCs' room temperature saturation magnetizations are M_S= 71.2 and 73.4 emu/g for the 50.2 nm and 85.6 nm CNCs samples, respectively.

The Larmor frequency shift, $\Delta\omega$ is calculated by the formula:

$$\Delta\omega = \frac{\gamma\mu_0 M_V{}^*}{3} \tag{3}$$

where γ= 2.67513× 10^8 rad $s^{-1}T^{-1}$ is the proton gyromagnetic ratio and μ_0= 4π×10^{-7} T m A^{-1} the magnetic permeability of the vacuum.

The estimated $\Delta\omega$ for the 50.2 nm and 85.6 nm CNCs samples are 2.37 ×10^7 s^{-1} and 2.95 ×10^7 s^{-1}, respectively.

The translational diffusion time τ_D is given by the formula:

$$\tau_D = \frac{D_{hydro}^{\ 2}}{4D} \tag{4}$$

where D_{hydro} is the hydrodynamic diameter of the CNCs, as calculated from the Dynamic Light Scattering (DLS) experiments and D= 3 ×10^{-9} m^2/s the water-proton translational diffusion time .

The calculated values of the τ_D are 0.2 and 0.6 μs for the 50.2 nm and 85.6 nm CNCs samples, respectively.

Based on the magnitude of the parameters derived from equations (3) and (4), $\sim 5 < \Delta\omega\ \tau_D < \sim 20$ for the CNCs (Table S1). For this reason we are of the opinion that the transverse relaxivity (r_2) values for both CNCs samples can be predicted by models assuming that the spin-spin relaxation occurs in the static-dephasing-regime (SDR).

Table S2. Compilation of in-vitro transverse relaxivities, r_2, that allow comparison of the maghemite nanoclusters (CNCs) developed herein against other nanoarchitectures with potential in MR imaging. The materials were divided in two major categories depending on the nature of their magnetic state, namely, superparamagnetic (SPM) and ferrimagnetic (FiM). The regime, SDR or MAR, based on the outer sphere relaxation theory, in which they fall in is shown. The frequency, ν [$= (\gamma_\mu/2\pi)$ H], at which the relaxivity was measured, is also given.

Magnetic State	**Type of nanoarchitecture**	**Sample surface functionalisation**	**Regime**	**r_2 ($mM^{-1}s^{-1}$)**	**ν (MHz)**
SPM	*Individual*	NCs capped with polymer [3]	MAR	11	60
		NCs capped with dendrons [4]	MAR	349	64
		NCs capped with DHAA [5]	MAR	121	64
		Endorem[a]	SDR	99	64
	Encapsulated in matrices	NCs encapsulated in Dextran [6]	SDR	312	64
		NCs encapsulated in liposomes[7]	SDR	116	20
	Clusters	Citrate-stabilized multi-core particles [8]	SDR	365	9.25
		PVP-stabilized nanospheres [9]	SDR	94	64
		polystyrene-capped nanoclusters [10]	SDR	435	128
		Dextran-coated nanoclusters [6]	SDR	312	64
		PEG-stabilized nanoflowers [11]	SDR	238	64
		(Mal)mPEG-PLA copolymer-capped nanoclusters[12]	SDR	465	60
		amine-stabilized nanoassemblies [13]	SDR	315	85
		TREG-stabilized nanoclusters [14]	SDR	295	400
		34 nm and 63 nm PAA capped nanoclusters [15, 16]	SDR	540 630	64
FiM	*Individual*	nanocubes encapsulated in PEG-phospholipids our system [17]	SDR	761	128
	Encapsulated in matrices	liposome-encapsulated magnetic nanocluster [18].	SDR	1286	100
	Nanocrystal assemblies [a]	PAA capped 50 nm nanoclusters[a]	SDR	405	60
		PAA capped 86 nm nanoclusters[a]	SDR	508	60

[a] results of the present work

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