Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Materials

Hybrids of manganese oxide and lipid liquid crystalline nanoparticles (LLCNPs@MnO) as potential Magnetic Resonance Imaging (MRI) contrast agents

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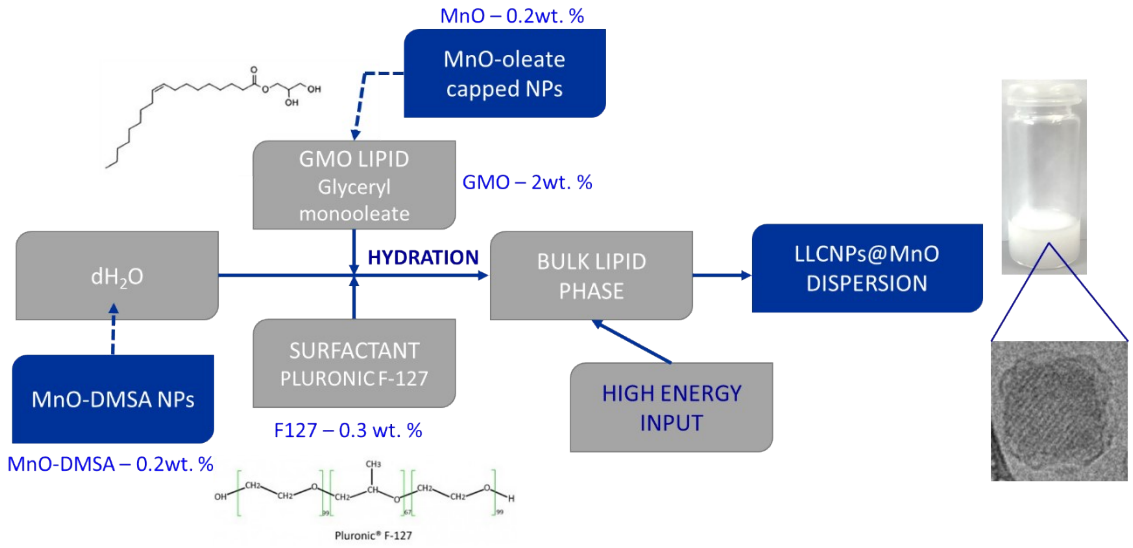

Figure S1 Schematic representation of the preparation of unloaded and MnO-loaded LLCNPs (with MnO-oleate capped and MnO-DMSA) using the top-down approach.

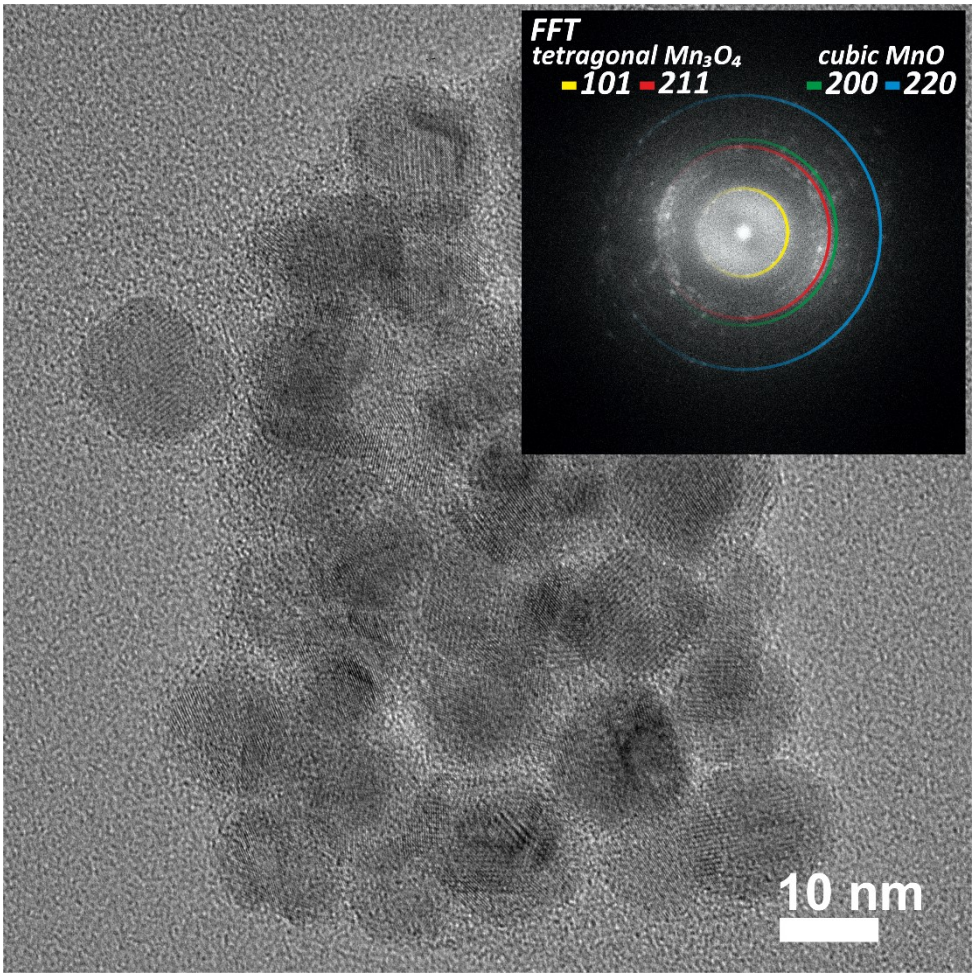

Figure S2 High resolution transmission electron microscopy (HRTEM) image of agregated MnO-DMSA NPs. In the inste FFT difractogram from the image, including all paricles, is presented. The rings – yellow, red, green and blue dnenote diffraction rings characteristic for 101, 211 of Mn_3O_4 tetragonal structure and 200, 220 of MnO cubic phase, respectively.

Table S1 Summary of full DLS results of prepared nanoparticle size (Z-ave – cumulant mean, $d_{Intensity}$ - intensity distribution, d_{Number} – number distribution, PdI – polydispersity index) and zeta potential

Sample	Z-ave / nm	SD	$d_{Intensity}$ / nm	SD	d_{Number} / nm	SD	PDI	SD	Zeta /mV	SD
MnO- oleate-capped#	74.6	0.30	83.6	1.10	15.9	3.00	0.308	0.035	-31.1	14.20
MnO-DMSA	182.9	2.35	209.8	10.80	120.4	18.15	0.148	0.010	-14.2	0.51
MnO-DMSA_1m	185.0	17.59	239.7	15.42	132.6	16.03	0.255	0.076	-11.2	0.93
MnO-DMSA_HSA	12.1	1.75	4.6 (53.4)*	0.13	3.6	0.16	0.553	0.006	-3.6	0.17
			313.0 (38.3)*	106.40						
LLCNPs	148.2	2.07	175.9	9.94	82.9	13.10	0.172	0.016	-19.8	0.60
LLCNPs _1m	160.2	0.60	193.8	7.80	64.6	38.00	0.162	0.032	-17.8	0.56
LLCNPs _HSA	8.6	0.20	4.6 (62.9)*	0.10	3.4	0.10	0.440	0.009	-1.6	0.86
			234.5 (35.9)*	11.75						
LLCNPs _NR	144.3	0.61	178.5	3.43	77.89	5.20	0.195	0.007	-20.9	1.04
LLCNPs@MnO-oleate	220.6	1.63	246.0	6.33	167.8	6.63	0.138	0.023	-20.2	1.21
LLCNPs@MnO-oleate _1m	410.1	12.38	500.3	27.69	242.4	6.65	0.297	0.032	-28.5	0.61
LLCNPs@MnO-oleate _HSA	13.0	0.30	447.5 (51.9)*	47.10	3.5	0.09	0.656	0.012	-0.8	0.02
			4.5 (48.1)*	0.05						
LLCNPs@MnO-DMSA _NR	137.4	0.76	162.4	6.50	68.10	23.21	0.158	0.001	-18.0	0.86
LLCNPs@MnO-DMSA	144.4	1.98	170.6	5.75	78.1	12.63	0.162	0.014	-16.2	1.17
LLCNPs@MnO-DMSA _1m	182.6	3.60	202.7	4.90	143.1	3.30	0.102	0.012	-21.2	2.08
LLCNPs@MnO-DMSA _HSA	9.5	0.10	4.6 (59.5)*	0.06	3.4	0.01	0.484	0.006	-3.0	0.85
			240.2 (36.7)*	7.44						
LLCNPs@MnO-DMSA _NR	143.4	0.78	166.0	5.58	88.66	5.93	0.158	0.012	-17.6	1.30
HSA	10.7	0.10	4.8 (51.9)*	0.04	3.5	0.10	0.501	0.004	-9.0	0.52
			88.3 (46.7)*	1.76						

including standard deviation (SD, n=3).

*bimodal PSD, values in brackets are the area under PSD peaks; #measurements in hexane

Table S2 Summary of DLS results of nanoparticle size (Z-ave - cumulant mean, $d_{Intensity}$ - intensity distribution, d_{Number} - number distribution, PdI - polydispersity index) and zeta potential including standard deviation (SD) for samples previously incubated in cell culture DMEM medium (Dulbecco's modified Eagle's medium) for 24 h. For measurements new sample batches were used.

Sample	Z-ave / nm	SD	$d_{Intensity}$ / nm	SD	d_{Number} / nm	SD	PDI	SD	Zeta / mV	SD
MnO-DMSA	166.8	2.56	186.0	8.39	107.7	9.33	0.179	0.020	-16.2	0.86
MnO-DMSA_DMEM_24h	500.8	18.60	588.5	8.65	372.1	166.90	0.269	0.026	-19.4	0.50
LLCNPs	161.9	4.26	181.8	2.82	57.3	20.05	0.252	0.015	-22.9	0.87
LLCNPs_DMEM_24h	152.5	1.32	195.5	5.58	40.9	18.36	0.343	0.010	-17.2	0.49
LLCNPS@MnO-oleate	282.4	1.83	332.1	19.70	212.8	5.03	0.188	0.007	-18.5	0.57
LLCNPS@MnO-oleate_DMEM_24h	219.6	5.22	311.1	12.52	81.73	75.29	0.275	0.008	-33.5	1.57
LLCNPs@MnO-DMSA	142.8	2.28	166.6	4.75	90.7	2.51	0.135	0.008	-18.2	0.40
LLCNPs@MnO-DMSA_DMEM_24h	137.0	2.05	169.3	0.51	47.5	26.42	0.182	0.004	-18.6	1.15

Table S3 The list of primers used in the gene expression studies.

Gene	Primer Forward	Primer Reverse	Amplicon size
GAPDH	5'-AAGGTCGGAGTCAACGGATTT-3'	5'-ACCAGAGTTAAAAGCAGCCCTG-3'	66bp
ACTB	5'-GCTCTTTTCCAGCCTTCCTT-3'	5'-CATACAGGTCTTTGCGGATGT-3'	106bp
ACTG1	5'-CCGAGCCGTGTTTCCTTCC-3'	5'-GCCATGCTCAATGGGGTACT-3'	142bp
PFN1	5'-GGGTGGAACGCCTACATCG-3'	5'-CCATTCACGTAAAAACTTGACCG-3'	182bp
LMNA	5'-AATGATCGCTTGGCGGTCTAC-3'	5'-CACCTCTTCAGACTCGGTGAT-3'	93bp
LMNB2	5'-GTCCTGGATGAGACGGCTC-3'	5'-GCGCTCTTGTTGACCTCGT-3'	89bp
TUBA4a	5'-TGAGATCCGAAATGGCCCATA-3'	5'-TAGTGACCACGGGCATAGTTG-3'	96bp

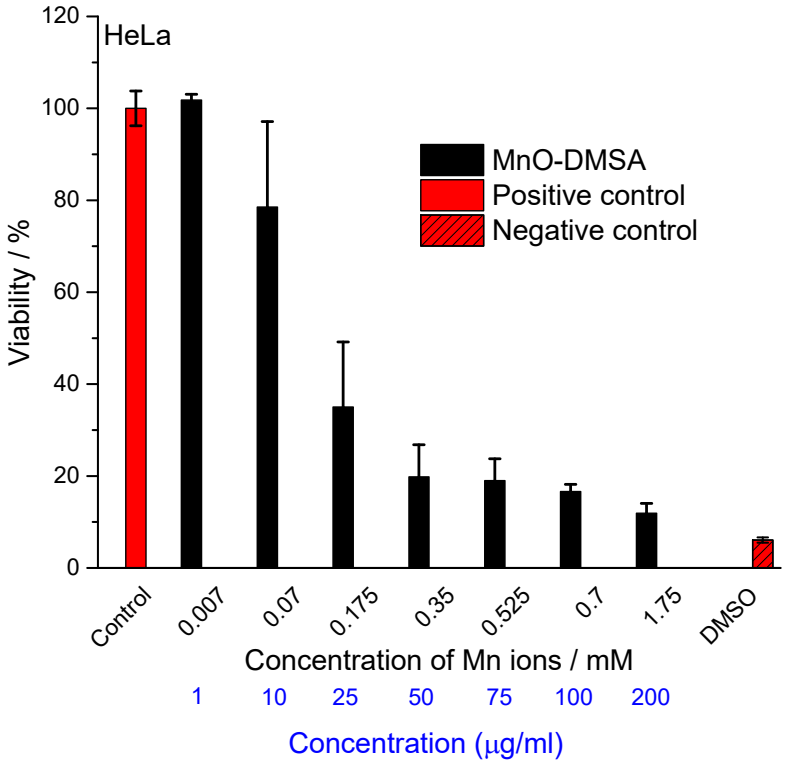

Figure S3 Viability studies in HeLa cells monolayers showing cytotoxic efficacy of MnO-DMSA in comparison to positive (DMSO) and negative control (non-treated cells), as a function of **NPs (μg/ml)** and **Mn ions concentration (in mM)**. The n-fold viability was measured by WST-1 assay (n=3). Based on these preliminary results the "safe" Mn ions concentration range, which was further applied in relaxivity was falling within the range 0.01 – 0.1 mM for MnO-loaded LLCNP.

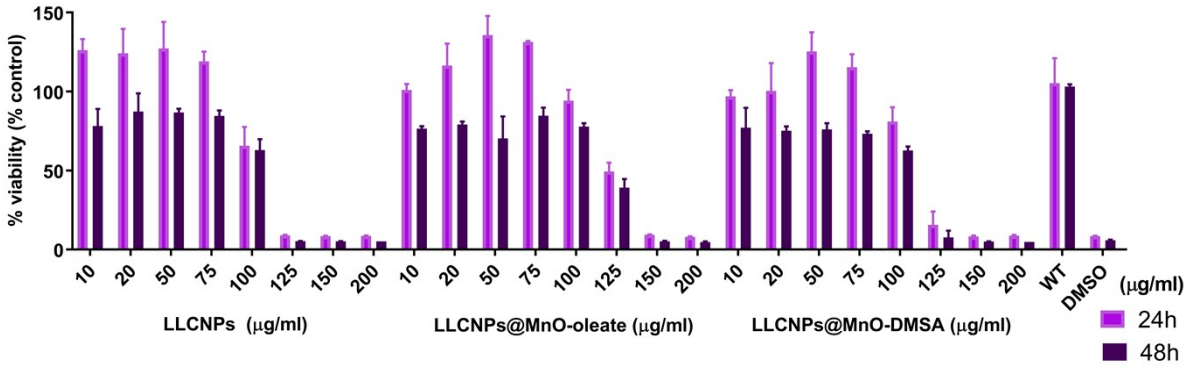

Figure S4 Viability of normal human fibroblast (MSU1.1 cells) after 24 h and 48 h of incubation with different concentrations (10, 20, 50, 75, 100, 125, 150, 200 μg/mL) of prepared LLCNPs, LLCNPs@MnO-oleate and LLCNPs@MnO-DMSA, investigated with WST-1 assay. Control is cells non-treated, and DMSO is cells treated with 50% v/v DMSO. Data are given as mean ± SD for three replicates.

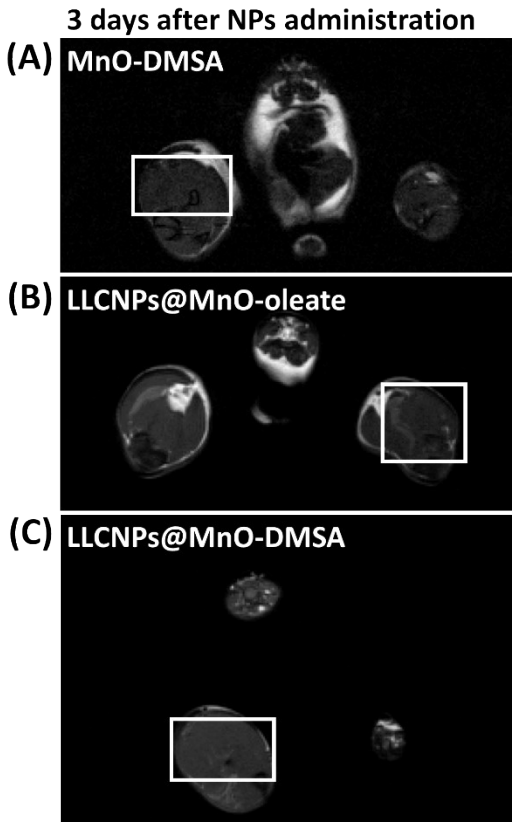

Figure S5 *In vivo* T_1-weighted MR images recorded 3 days after administration of: **(A)** MnO-DMSA, **(B)** LLCNPs@MnO-oleate, **(C)** LLCNPs@MnO-DMSA. The images were collected with a 9.4 T horizontal MRI scanner, at 37°C. Marked with a white squares are regions of hind limbs with 3 days earlier administered NPs as contrast agent – no contrast enhancement is observed.