Supporting information

Bioinspired, Manganese-Chelated Alginate-Poly (Dopamine) Nanomaterials for Efficient *in Vivo* T_1-Weighted Magnetic Resonance Imaging (MRI)

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EXPERIMENT

A. Xylenol orange disodium salt for spectrophotometric determination of free manganese ions.

A xylenol orange disodium salt (3, 3′-bis [N,N-bis(carboxymethyl)aminomethyl]-o-ocresolsulfonephthalein tetrasodium salt) acetic acid buffer (pH 5.8) and calibration curve was obtained according to previously report [5]. For determination of manganese ions, 100 µL of sample was added to 2 mL of xylenol orange disodium salt acetic acid buffer prior to UV detection at 576 and 434 nm. The quantity of free manganese ions was calculated based on the fitted equation.[7] The leached manganese cation from AlgPDA(Ca/Mn) in vivo conditions was mimicked by estimating of the amount of Mn cation release in phosphate buffer at 37 °C under sink environments (20 mL) by continuous stirring over a period of 4 hours . To evaluate the amount of leached manganese ions, spectroscopy quantification was performed using the xylenol orange indicator at 0.5hr, 1hr, 2hr and 4hrs intervals. The absorbance of free Mn cation leakage was evaluated at 434 and 576 nm.

B. PEG surface coating to form AlgPDA(Ca/Mn)-PEG preparation

20 mg (92µmole of alginate repeat) was dissolved in 10ml of 0.1Mes buffer (pH=5.9). Next, 354.64 µg (1.85 µmole) of EDC and 401.69 µg (1.85 µmole) of NHS were added for activation. Then, 18.5mg (1.85 µmole) of O-(2-Aminoethyl) polyethylene glycol (HO-PEG-NH2) was added into AlgPDA(Ca/Mn) under continuous stirring in a room temperature. The mixture was proceed for 24hrs to initiate the coupling chemistry after adjusting its pH (8.0) using Tris-HCl buffer (50 mM). Finally, the resulting product was extensively purified by dialysis membrane against distilled water using dialysis bag (MWCO= 12-14kDa), followed by lyophilization to obtain. AlgPDA(Ca/Mn)-PEG nanoparticles.

Table S1. Particle size and Zeta potential of synthesized nanogels

Sample	DLS particle size (nm)	PDI	Zeta potential (mV)
AlgPDA(Ca)	171.93 ± 59.12	0.14 ±7.6	−66.4 ±4.6
AlgPDA(Ca/Mn)	66.30 ±6.5	0.13 ±0.21	−24.26 ± 2.8
AlgDA(Ca)	532.83 ± 18.27	0.79 ±0.34	−66.33 ± 3.1
AlgDA(Ca/Mn)	135.24 ±4.5	0.64 ±0.14	−27.7 ± 3.5

Table S2. Spectroscopic determination of Mn release from AlgPDA(Ca/Mn) by xylenol orange dye. Ratio of absorbance ratio at 576 and 434 nm and approximate release of Mn in milimolar.

Time	Absorbance ratio 576/434			Mn released in mM		
	pH= 5.0	pH=6.5	pH= 7.4	pH= 5.0	pH=6.5	pH= 7.4
0.5hr	0.489	0.341	0.331	0.245	-	-
1hr	0.495	0.381	0.331	0.258	0.033	-
2hr	0.530	0.415	0.378	0.327	0.09	0.02
3hr	0.539	0.411	0.377	0.344	0.09	0.02
4hr	0.556	0.412	0.377	0.378	0.094	0.02

Table S3. *In vivo* bio-distribution of Mn at various time points in major organs (4 hours, 24 hours,5 days, and 16 days) after injection AlgPDA(Ca/Mn) NGs and control without injection of the contrast agent . based on triplicate measurements

Organ	Mn Mean (ppb) with standard deviation				
	Control	4 hour	24 hour	5 days	16 days
Kidney	25.72 ± 5.04	194.5 ±6.34	66.62±5.4	127.2±6.2	45.2±5.31
Liver	49.84 ± 5.5	221.2±6.1	222.3±5.9	171.1±5.8	65.76±5.21
Spleen	28.33 ± 5.7	37.15±6.05	54.18±6	22.68±6.4	10.9±5
Tumour	7.909 ± 5.8	32.29±6.45	140.9±6.5	24.21±6	45.23±5.8

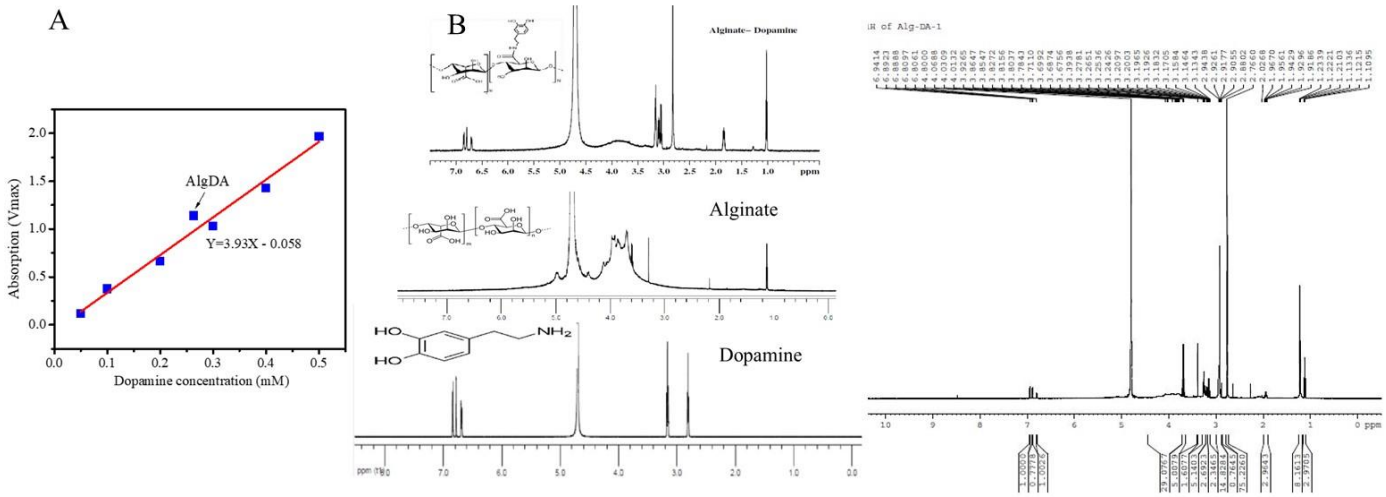

Figure S1. (A) Standard curve showing Dopamine (DA) conjugation with alginate (Alg) to form AlgDAat 280 nm (B) ^{1}H-NMR Spectra of analysis confirmed AlgDA conjugation, NMR analysis showed that the peaks of the dopamine protons appeared at around 7 ppm: solvent, D2O

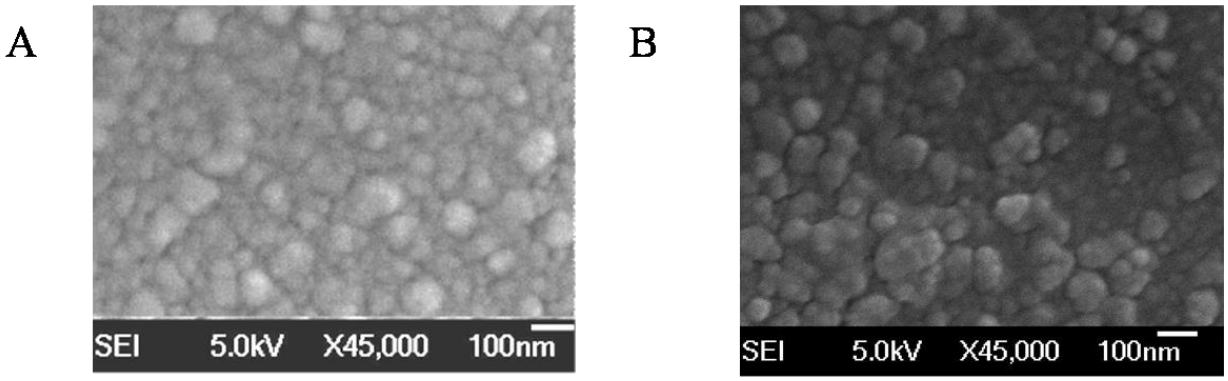

Figure S2. SEM image of a) algPDA(Ca/Mn) (b) AlgDA(Ca/Mn)

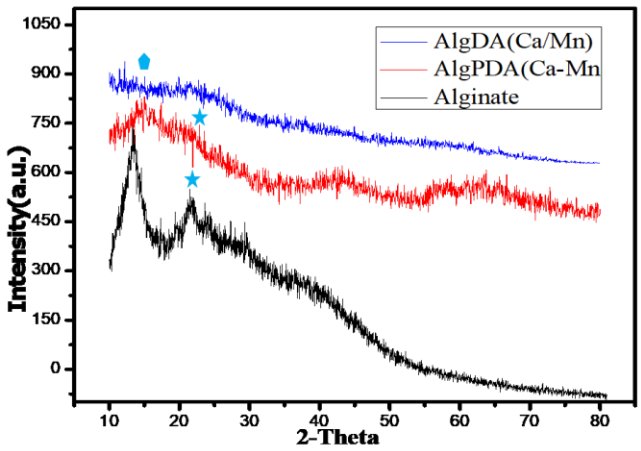

Figure-S3. XRD patterns of AlgDA(Ca/Mn), AlgPDA(Ca/Mn) and Alginate(Alg)

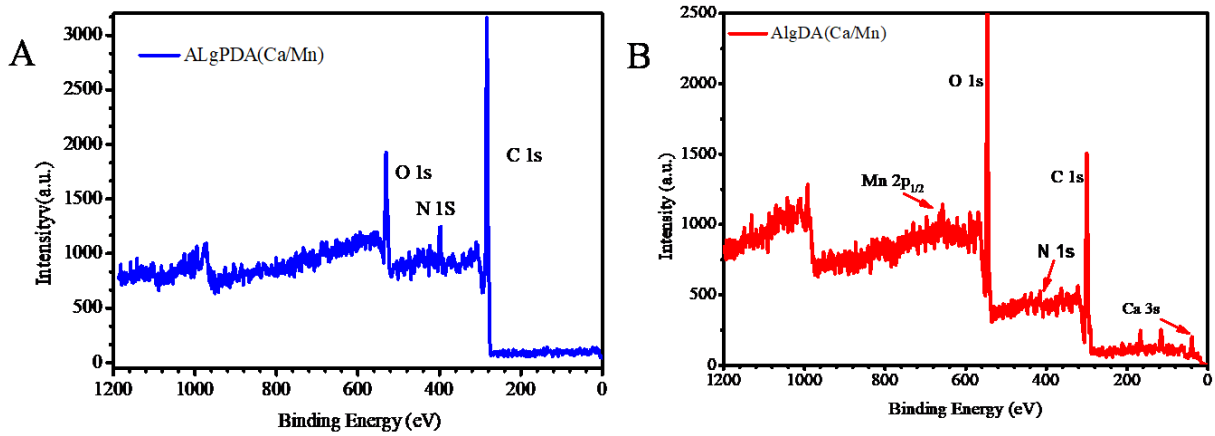

Figure-S4. Surveys spectra of (A) AlgPDA(Ca/Mn) (B) AlgPDA(C/Mn) NGs

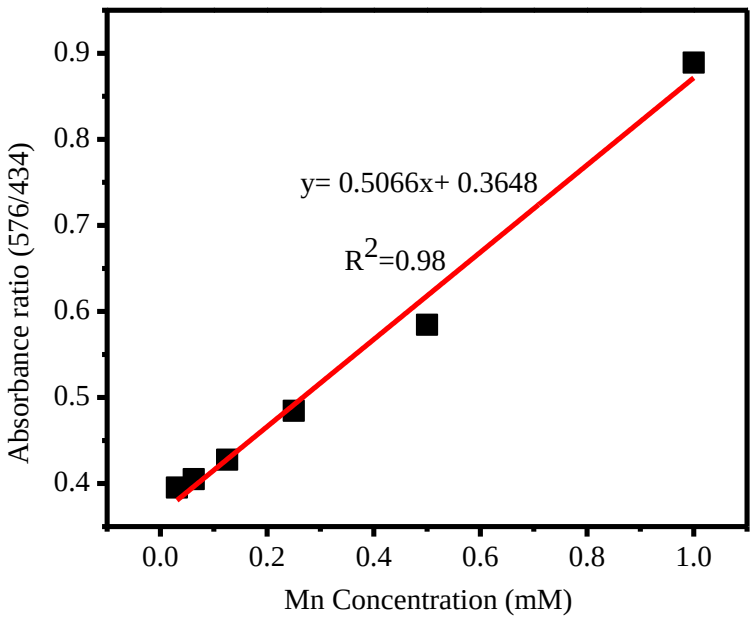

Figure S5. Spectroscopic determination of Mn release by xylenol orange dye: absorbance vs concentration. The calibration curve was obtained from standard free Mn cation (black) square).

Grafting of ALGPDA(Ca/Mn) nanoparticles with polyethylene glycol:

PEGylated ALGPDA(Ca/Mn) nanogels were modified on to the carboxyl groups of the alginate AlgPDA and amino groups of the polyethylene glycol (HO-PEG-NH2). As we can see figure-S5A, the absorbance of the [C–O–C] stretching vibration at 1100 cm^{-1}, confirming the successful conjugation of PEG chains [1]. Moreover, absorbance between 2850-2940 cm^{-1} corresponding to [-CH_2-] indicates the addition of PEG [8]. These reactions were also confirmed by ^{1}H NMR spectrum analysis (Figure S5B). The ^{1}H NMR spectrum of the ALGPDA(Ca/Mn)-PEG showed the chemical shifts of PEG at δ = 3.75 and for AlgPDA δ = 2.76 and 1.2 could be seen in the ^{1}H NMR spectrum of the ALGPDA(Ca/Mn)-PEG nanogels, hence, confirming PEG is successfully to the functional ALGPDA(Ca/Mn) nanogels [2].

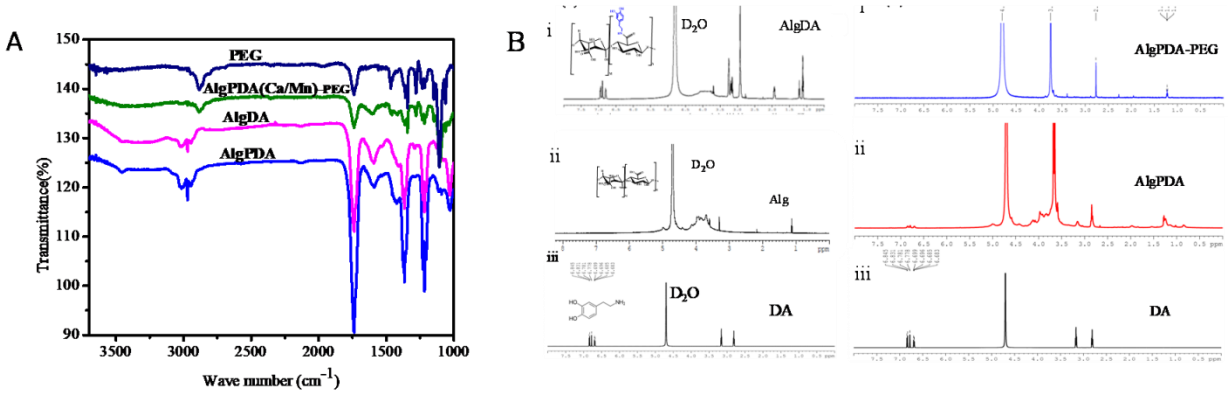

Figure-S6. Characterization of PEG surface coating to form AlgPDA(Ca/Mn)-PEG preparation. (A) FTIR spectra of PEG, AlgPDA(Ca/Mn)-PEG, AlgDA and AlgPDA . (B) 1H NMR Spectra analysis confirmed PEG surface coating to form AlgPDA(Ca/Mn)-PEG preparation (b) The 1H NMR spectrum of DA, Alg, AlgDA, AlgPDA and AlgPDA (Ca/Mn)-PEG: solvent, D2O, Abbreviations: DA, Dopamine; Alg, Alginate; AlgDA, Alginate-dopamine; AlgPDA, Alginate-polydopamine

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1. Mekuria, S. L.; Debele, T. A.; Tsai, H. C., Encapsulation of Gadolinium Oxide Nanoparticle (Gd2O3) Contrasting Agents in PAMAM Dendrimer Templates for Enhanced Magnetic Resonance Imaging in Vivo. *ACS Appl Mater Interfaces* **2017,** *9* (8), 6782-6795.
2. Jia, X.; Pei, M.; Zhao, X.; Tian, K.; Zhou, T.; Liu, P., PEGylated Oxidized Alginate-DOX Prodrug Conjugate Nanoparticles Cross-Linked with Fluorescent Carbon Dots for Tumor Theranostics. *ACS Biomaterials Science & Engineering* **2016,** *2* (9), 1641-1648.