Electronic Supplementary Information (ESI†)

Polyaniline shell cross-linked Fe_3O_4 magnetic nanoparticles for heat activated killing of cancer cells

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Cell Culture:

Mouse Skin Fibrosarcoma (WEHI-164) cell line was obtained from National Centre for Cell Sciences, Pune, India. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM: GIBCO, Invitrogen, Carlbad, CA, USA) supplemented with 10% fetal calf serum (FCS: Himedia Laboratories, Mumbai, India) and antibiotics (100 U ml^{-1} penicillin and 100 µg ml^{-1} streptomycin) in a humidified atmosphere of 5% CO_2 at 37 °C. The desired number of cells were seeded in complete DMEM and incubated at culture conditions for overnight, followed by treatment with magnetic nanoparticles.

MTT assay:

After exposure of AC magnetic field to the PSMN treated cells, cells were cultured for 48 h. Then, the media containing PSMN was carefully removed and the cells were further incubated with 0.5 ml of MTT solution (0.5mg/ml, Sigma, USA) at culture conditions for 2 h. The supernatant was aspirated and 1 ml of DMSO was added to each culture dish to solubilize the MTT crystals. The crystals were thoroughly dissolved and further diluted (1: 10) with dimethyl sulfoxide (DMSO). 200 µl of above solution from P-60 culture dishes was transferred to 96 well plates and the blue colour was read in a microplate reader (Tecan infinite 200 PRO, Switzerland)

at 544 nm. The cell viability was calculated by comparing the absorption of treated cells to that of control, which was defined as 100%.

Results and discussion:

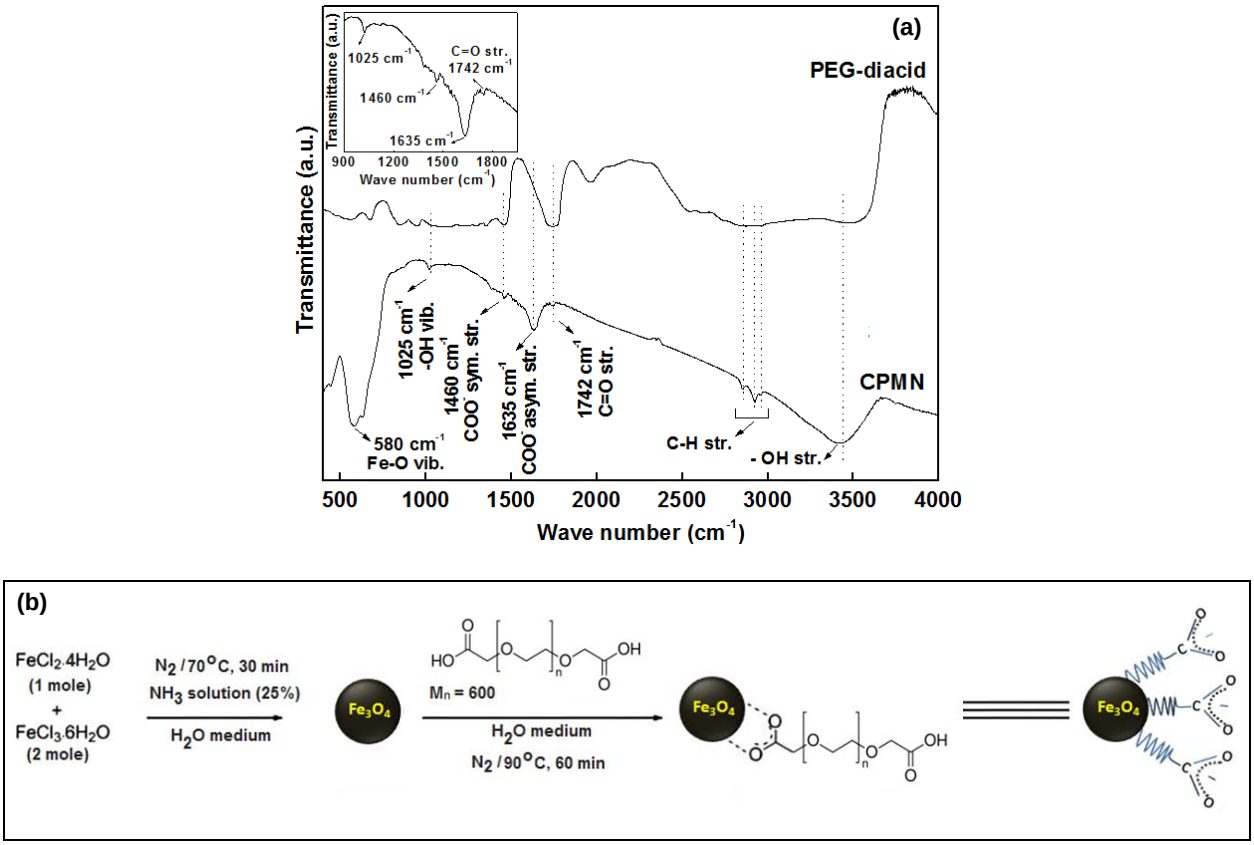

Fig. S1. (a) FTIR spectra of PEG-diacid and CPMN (inset shows the FTIR spectra of PEG-diacid in the region of 900-1900 cm^{-1} revealing the appearance of C=O stretching vibration) and (b) schematic representation of the synthesis of CPMN.

The absorption bands for the PEG-diacid (HOOC-PEG-COOH) are well resolved, but those of the CPMN are rather broad and few. The characteristic C=O band at 1742 cm^{-1} for PEG-diacid is significantly reduced and an additional intense band appeared to a lower wave number, 1635 cm^{-1} in the spectra of the CPMN. This spectral change indicates the binding of PEG-diacid to surface of Fe_3O_4 nanoparticles by chemisorptions of carboxylate ions (Fig. S1b). Some of the carboxylate group of PEG-diacid form complexes with Fe atoms on the surface of Fe_3O_4 rendering partial single bond character to the C=O bond. This results in weakening of C=O bond,

which shifts the stretching frequency to a lower value. However, the retention of C=O vibration mode at 1742 cm^{-1} (weak signal as compared to PEG-diacid) in CPMN suggests the presence of free carboxyl groups on the surface of Fe_3O_4 nanoparticles. Further, the vibrational modes appeared at 1460 and 1025cm^{-1} in CPMN correspond to the CH_2 scissoring and carboxylic -OH group of PEG-diacid. Earlier investigation on conjugation of Fe_3O_4 nanoparticles with PEG-diacid also suggested that one carboxylate head is the preferable site for chemical conjugation with Fe_3O_4 leaving the other one free.[1,2] The strong IR band observed at around 580 cm^{-1} in CPMN can be ascribed to the Fe-O stretching vibrational mode of Fe_3O_4.

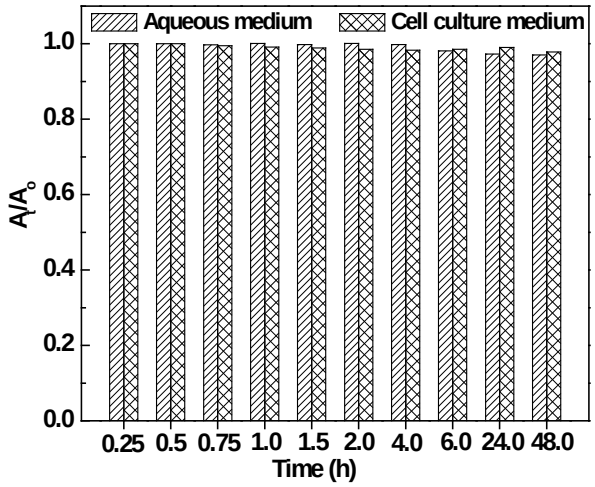

Fig. S2. Normalized UV absorbance (A_t/A_0) vs. time plot of CPMN (0.1 mg/ml) at wave length of 350 nm in aqueous and cell culture medium (A_t = absorbance at time 't' and A_0 = Absorbance at t = 0).

The colloidal stability of the CPMN was also assessed from the extinction changes with time. The insignificant change in absorbance of CPMN suspensions in aqueous and cell culture media (DMEM supplemented with 10 % FCS) indicates their good colloidal stability. Thus, it can be proposed that the PEG-diacid moieties on surface of Fe_3O_4 make these particles hydrophilic by formation of hydrogen bonds between surface functional groups (free -COOH) and water. In addition, the electrostatic repulsive force originating from the ionization of the surface groups provide additional stability to the particles.

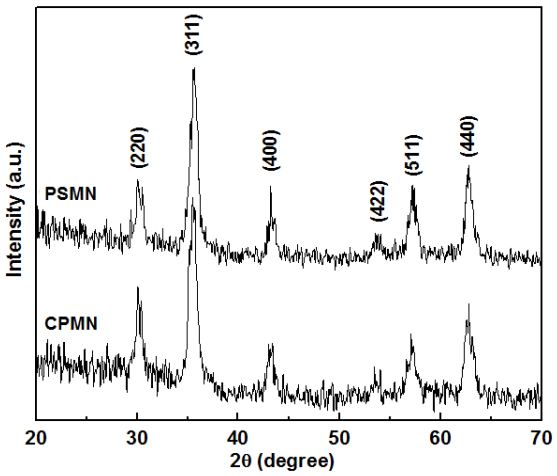

Fig. S3. XRD patterns of CPMN and PSMN.

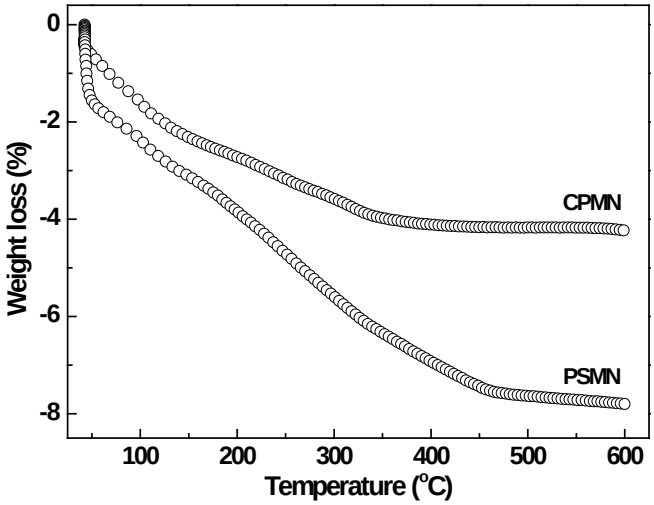

Fig. S4. TGA plots of CPMN and PSMN.

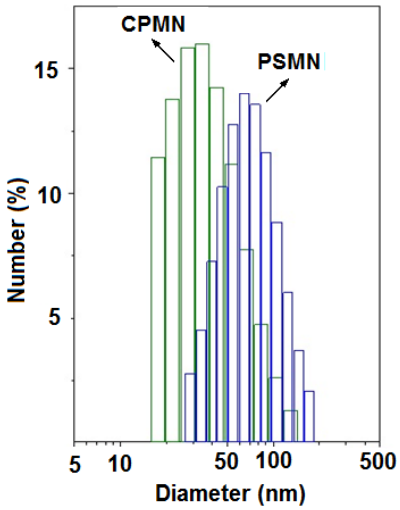

Fig. S5. DLS plots of CPMN (green colour) and PSMN (blue colour).

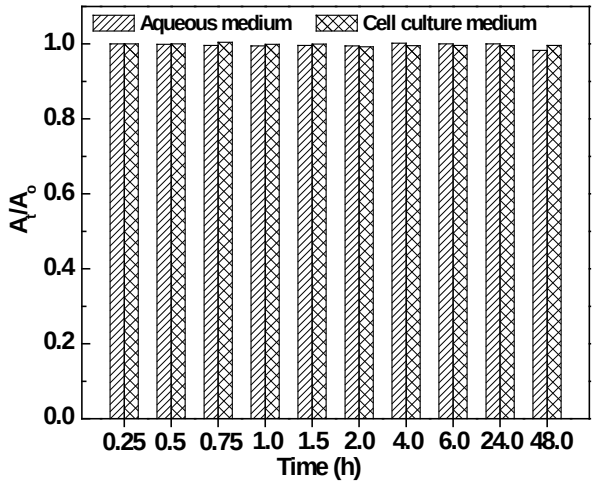

Fig. S6. Normalized UV absorbance (A_t/A_0) vs. time plot of PSMN (0.1 mg/ml) at wave length of 350 nm in aqueous and cell culture medium (A_t = absorbance at time 't' and A_0 = Absorbance at t = 0).

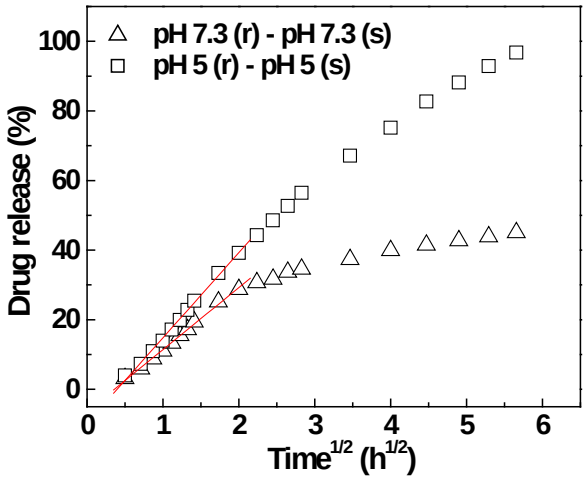

Fig. S7. Short time release behavior showing linear relationship between the drug release and square root of time.

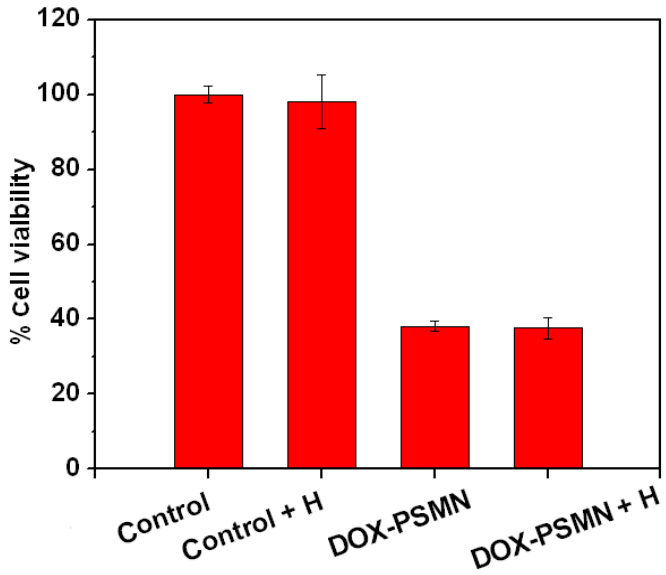

Fig. S8. Viability of WEHI-164 cells upon exposure of AMF (0.335 kOe) for 10 min in presence of DOX-PSMN (0.25 mg of PSMN having DOX concentration of 24 µM) with respective controls.

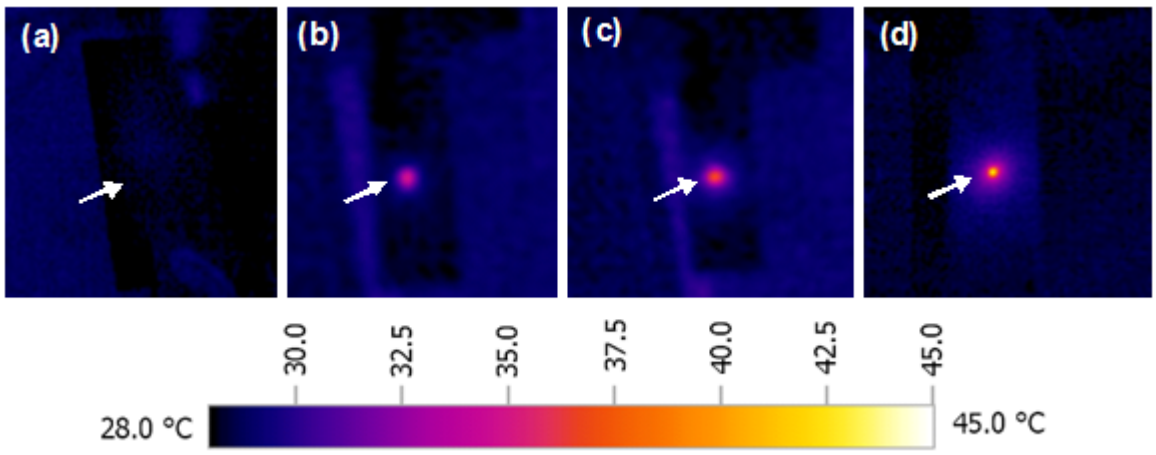

Fig. S9. Infrared thermal images of solid PSMN after exposing to laser light of 532 nm for (a) 0 min, (b) 5 min, (c) 8 min and (d) 15 min (arrow indicates the location of sample mounted on the glass slide).

Table S1. Zeta-potential values of PSMN after interaction with BSA protein.

PSMN (0.02 mg/ml) in 1 ml of 0.01 M PBS (pH 7.3)	PSMN (0.02 mg/ml) incubated with BSA (0.025 mg/ml) in 1 ml of 0.01 M PBS (pH 7.3)		
	30 min	1 h	2 h
-22.5	-22.3	-21.9	-21.8

References:

1. E. Occhipinti, P. Verderio, A. Natalello, E. Galbiati, M. Colombo, S. Mazzucchelli, A. Salvad, P. Tortora, S. M. Doglia and D. Prosperi, *Nanoscale*, 2011, **3**, 387.
2. F. Hu, K. W. MacRenaris, E. A. Waters, E. A. Schultz-Sikma, A. L. Eckermann and T. J. Meade, *Chem. Commun.*, 2010, **46**, 73.