Electronic supporting information

Biocompatibility, biodistribution and efficacy of magnetic nanohydrogels in inhibiting growth of tumor in experimental mice model

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Structural characterizations of the materials

FT-IR spectra

Fourier transform infrared spectra of poly(NIPAAm)-CS based hydrogels along with NIPAAm and chitosan (CS) were recorded in the range of 500 cm^{-1} to 4000 cm^{-1} through FTIR, Magna 550, Nicolet Instruments Corporation, USA using KBr pellet technique. It is shown in figure S1 (a).

The vinyl group >C=C< at 1620 cm^{-1} of NIPAAm monomer has disappeared from poly(NIPAAm) indicating the formation of long chain backbone along it. The amide I and amide II stretching at 1657 cm^{-1} and 1545 cm^{-1} remained the same. The vibration modes at 1150 cm^{-1} and 1080 cm^{-1} can be assigned to the asymmetric stretching of glycosidic linkage joining two monosaccharides and symmetric stretching of (C–O–C) of glucosamine unit associated with CS. The presence of characteristic band at 1610 cm^{-1} in CS indicates the incomplete deacetylation of chitin to chitosan. The band appearing at 1410 cm^{-1} can be assigned to methyl and methane bending vibration. The appearance of 3435 cm^{-1} band corresponds to vibration of intra and intermolecular H-bonded (–OH) groups.

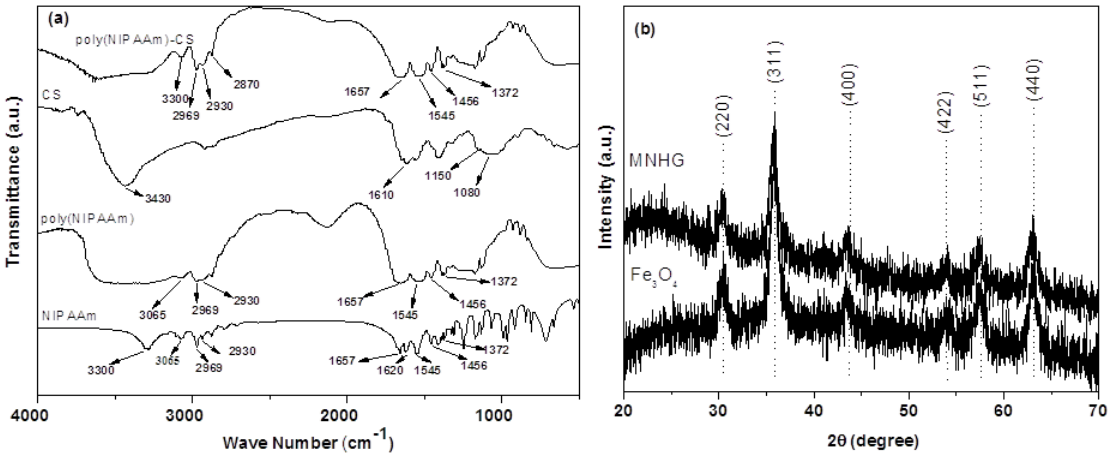

Fig. S1. (a) FT-IR spectra of poly(NIPAAm)-CS composite along with NIPAAm monomer indicating polymeirzation of NIPAAm as well as its grafting with chitosan (CS). (b) X-ray diffraction pattern of MNHG along with Fe_3O_4 NPs showing confirmation of presence of NPs into it as all the peaks indexed for magnetite are present in the MNHG as well.

Figure S1 (b) shows the X-ray diffraction pattern of MNHG along with Fe_3O_4 NPs recorded in the range (2θ) of 20 to 70° by X'Pert PRO, Philips, Netherlands using CuKα, λ = 1.5405 Å radiation. It shows the presence of all major characteristic peaks of magnetite as indexed in the diagram which confirms the presence of NPs within it.

Biocompatibility and biodistribution studies: protocol for measurements

The blood samples (~700 µL) were collected from each of the animals in sterilized glass vials containing 0.2% EDTA. The samples were analyzed for the biochemical and hematological parameters on the same day. Complete blood count (CBC) including examination of red blood cells (RBC), white blood cells (WBC), hemoglobin (HB), platelets (PLT), mean corpuscular hemoglobin (MCH), hematocrit (HCT) and mean corpuscular volume (MCV) were done in a semi-automated haematology analyzer BC 2300 (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, P.R.China) and differential leukocyte count (DLC) was done by examining blood smear microscopically. Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) enzyme activities were analyzed from the plasma using a fully automatic biochemistry analyzer, FA 200 (Clindiag systems BVBA, Belgium). Blood urea nitrogen (BUN) and creatinine levels were estimated using a semiautomatic biochemistry analyzer, ARX-100 (Micro Lab Instruments, India).

The vital organs from Dose I and Control groups were preserved in 70 % ethanol for NPs accumulation studies namely, analysis of magnetization by VSM and iron content through ICP-AES. For sample preparation for VSM, all the tissues were vacuum dried overnight at 50 ºC and then crushed to obtain powder. Almost all the amount was taken for magnetization measurements at room temperature using VSM (Model 7401, Lakeshore, USA). After VSM studies the same samples were completely dissolved in dilute HNO_3 solution under mild heat and used for iron concentration estimation through ICP-AES technique (Arcos, M/s. Spectro Germany).

Representative organs from all the three major experimental groups were preserved in 10 % formalin solution and the tissues were processed for routine H & E (hemotoxyline and eosin). Prussian blue staining was done on certain tissue samples to demonstrate accumulation of NPs.

Haematological data

Platelet counts

The 7 days observation for platelets after administration of dose I of MNHG (650 µg g^{-1}) showed significantly higher counts compared to their control levels inswiss mice. Therefore the extended analysis was carried out for 14 days post administration. The result is shown in figure S2. As can be seen the platelet counts parameterare returned its normal level after 14 days of observation.

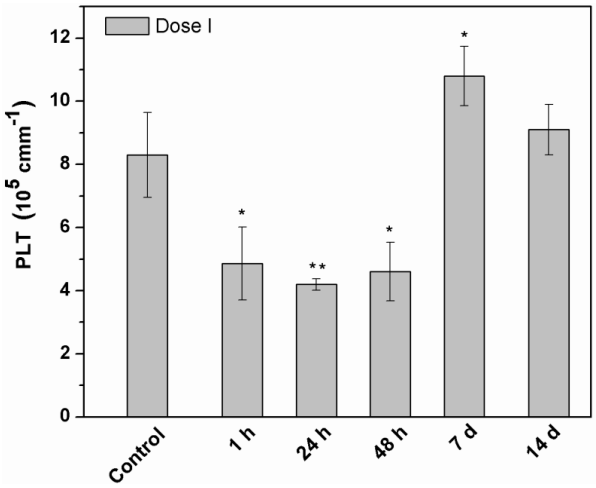

Fig. S2. The platelet count for dose I (650 µg g^{-1}) body wt. for 14 days. This extended study was carried out in the view of relatively significant values (p < 0.05) after 7 days of observation. Results are expressed as mean ± s.d. (n = 8). * p < 0.05 and ** p < 0.005 with respect to control. h= hour, d= day

Leukocyte counts

Different leukocyte counts (DLC) in blood are shown below. No significant changesin counts were found after 7 days post-MNHG administration

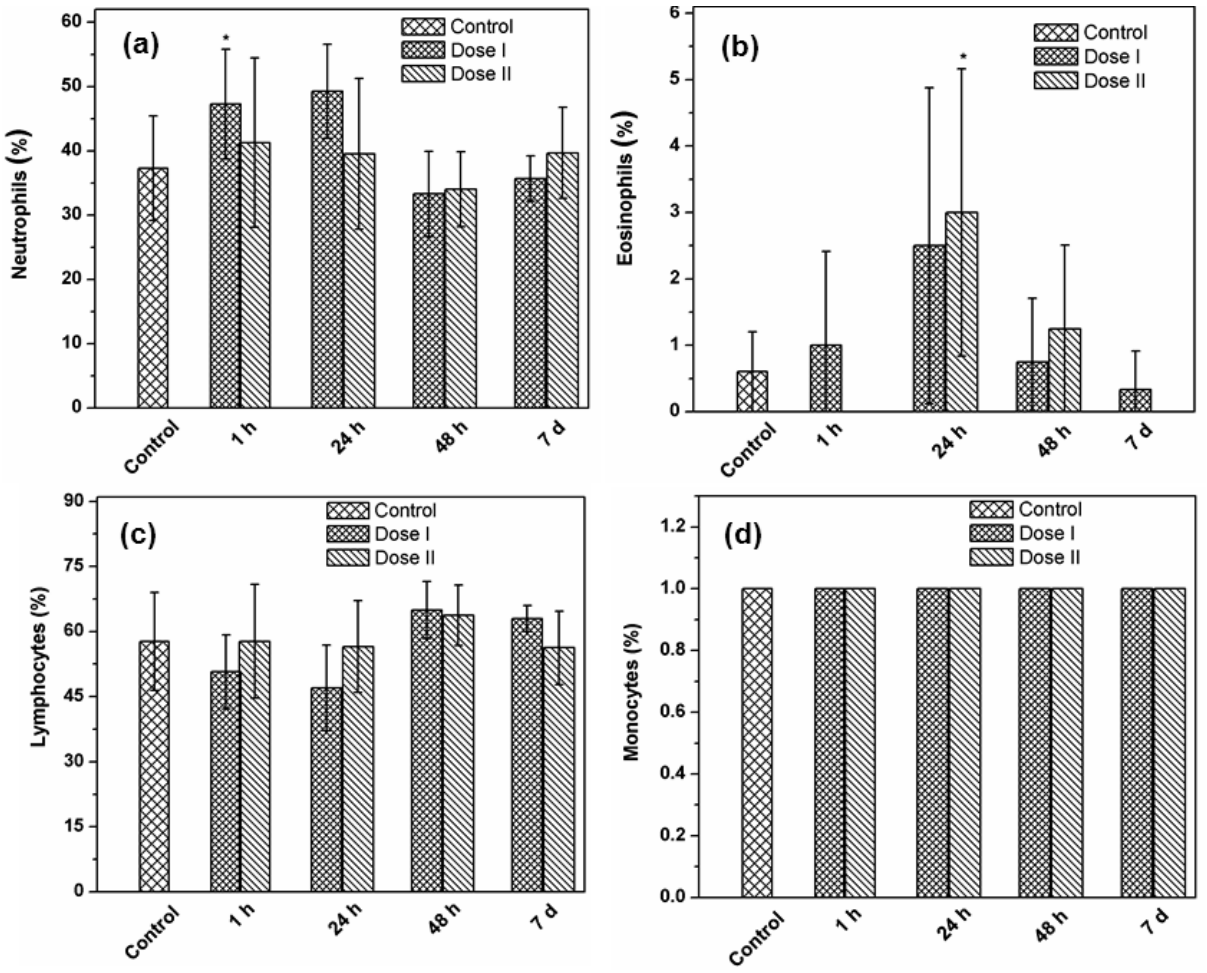

Fig. S3. Histograms showing differential leukocyte count (DLC) in SWISS mice at 1 h, 24 h, 48 h, and 7 d of i.v. administration of magnetic nanohydrogel (MNHG) at dosage 650 μg g^{-1} (Dose I) and 325 μg g^{-1} (Dose II) body wt. Results are expressed as mean ± s.d., (n = 8). Results are expressed as mean ± s.d. (n = 8). * $p < 0.05$ with respect to control. h= hour, d= day

Mean corpuscular volume (MCV) and Mean corpuscular haemoglobin (MCH) counts

Likewise, no change in the mean corpuscular haemoglobin concentration (MCHC) was detected during the study.

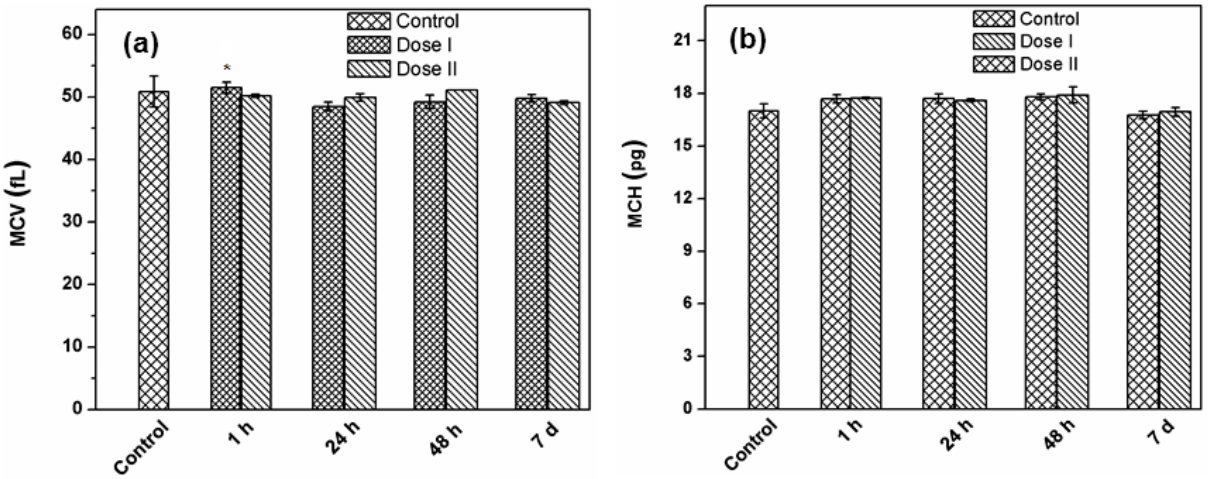

Fig. S4. Histograms showing (a) mean corpuscular volume (MCV) and (b) mean corpuscular hemoglobin (MCH) in SWISS mice at 1 h, 24 h, 48 h, and 7 d of i.v. administration of magnetic nanohydrogel (MNHG) at dosage 650 μg g^{-1} (Dose I) and 325 μg g^{-1} (Dose II). Results are expressed as mean ± s.d., (n = 8). Results are expressed as mean ± s.d. (n=8). * $p < 0.05$ with respect to control. fL= femtoliter and pg = picogram

Histopathology of vital organ tissues: H & E staining

Figure S5 shows representative photomicrographs of the hemotoxyline and eosin (H & E) stained tissue sections of lung, liver, spleen, kidney and brain of the mice taken at 7 d of i.v. administration of MNHG at 650 μg g^{-1} (Dose I). Gross and histological observations of all the vital organs were normal. All the tissues of MNHG treated animal showed normal architecture specific to the organs and there were no histopathological legions or abnormality in any of the sections processed for routine H & E staining. The overall microscopic impression indicates normal histophysiological status of the organs in the treated animals.

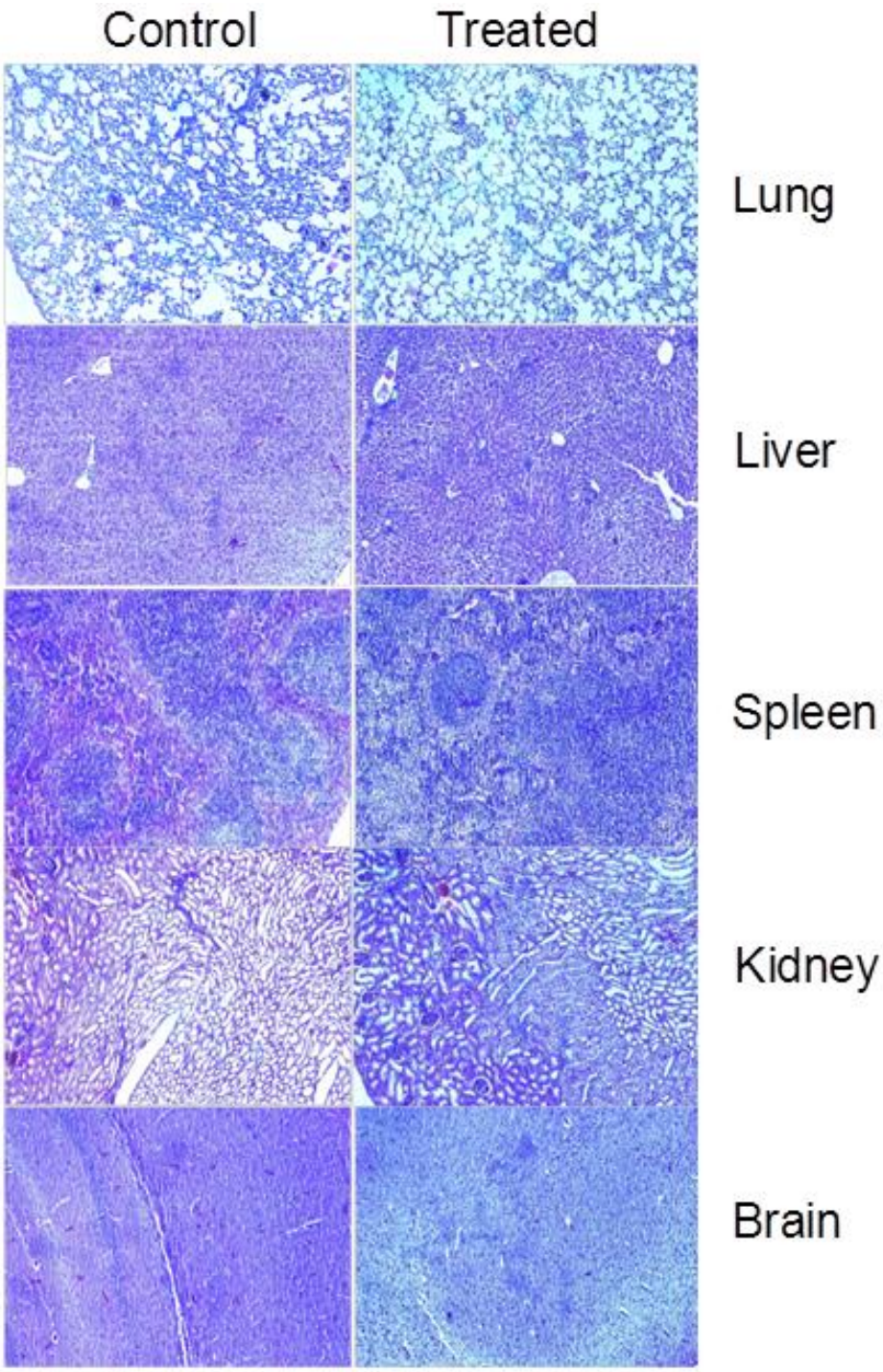

Fig. S5. H & E stained histological tissue sections at 10x under bright field image mode (a) lung, (b) liver,(c) spleen,(d) kidney and brain (e) tissue sections of mice on 7^{th} d after i.v. administration of magnetic nanohydrogel (MNHG) at 650 μg g^{-1} (Dose I).

Mice tumor surface temperature under AMF exposure

Figure S6 (a, b) shows the mean temperature on the surface of the tumor during the hyperthermia treatment after MNHG administration under AMF at fields 325 and 290 Oe respectively. Mean temperature recorded at multiple points on the tumor surface showed a gradual increase from nearly 33 to 35.5-38.5 °C at 10 min which ultimately reached to 38 - 40.4 °C after 20 min of AMF exposure at higher field (figure 6S a). The final tumor surface temperature was nearly 38 °C after 20 min of AMF exposure at 290 Oe (figure 6S b).

It is important to note that at field strength of 325 Oe, a single dose (650 μg g^{-1}) of MNHG could raise the surface temperature of tumor to 40 °C in 20 min. The MNHG samples were retained at the site of injection and were able to raise the temperature to 40 °C on subsequent AMF exposure of the tumor bearing animals at 72 h intervals. However, administration of a second dose of MNHG at an interval of 72 h had an additional advantage of raising and maintaining the temperature till 3rd treatment done after another 72 h of the previous field exposure.

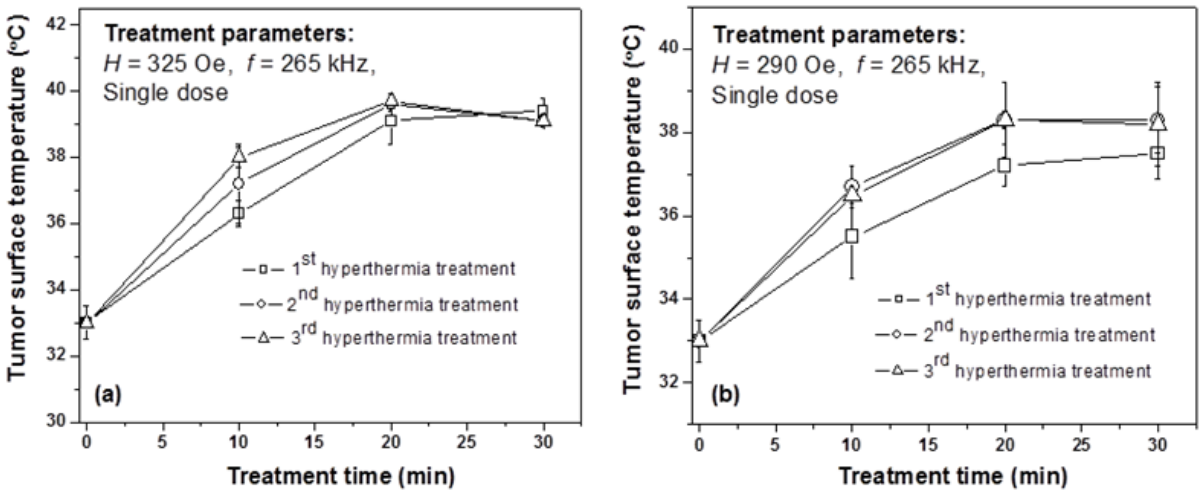

Fig. S6. Mean temperature on the surface of the transplanted fibrosarcoma tumor in mice during AMF exposures at field strength and frequency of (a) 325 Oe, 265 kHz and (b) 290 Oe, 265 kHz following single intratumoral administration of MNHG at 650 μg g^{-1} (Dose I). The mice were exposed to three hyperthermia treatments each at 72 h intervals.