Supporting information

Magnetic nanoscale metal organic frameworks for potential targeted anticancer drug delivery, imaging and MRI contrast agent

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Materials and Methods:

Paclitaxel loading and release study

Paclitaxel was loaded into nanoparticles by the dialysis method. Briefly, 20 mg of nanoparticles were dispersed in 4 mL of water/DMSO (2:8) mixture containing 5 mg of PTX. The mixture was sonicated for 3 min and then dialysed against 500 mL of water for 24 h at room temperature. The PTX loaded nanoscale magnetic MOF were separated by magnetic decantation. To remove the unloaded PTX, the nanoparticles are washed with PBS and magnetically concentrated. The solvents were then evaporated to allow encapsulation of PTX through porous shell and adsorbed into the magnetic nanoscale MOFs. Then the PTX loaded magnetic NMOFs were again dispersed in 4 mL of water/DMSO (2:8) mixture and particles were separated by external magnetic field. The amount of loaded PTX was measured by using high performance liquid

chromatography (HPLC, Aglient Technologies, USA). The mobile phase in a C18 column, consisting of acetonitrile/water (45:55, v/v) co solvent, with a flow rate of 1.0 mL/min. Eluted compounds were detected at 227 nm using a spectra 100 UV–Vis detector [1]. The PTX-loading amount was determined using a calibration curve of various PTX concentrations The drug loading content and entrapment efficiency were calculated according to very well-known formula.

$$\text{Drug loading contents (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of drug loaded nanoparticles}} \times 100$$

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of total drug injected}} \times 100$$

The release profile of PTX from the Fe_3O_4@IRMOF-3/FA nanoparticles was studied using a dialysis method [2]. 2 mg PTX loaded nanoparticles were dispersed in 10 ml PBS buffer and placed into a dialysis membrane with a molecular weight cut off (MWCO) of 14 kDa, then dialyzed against 100 mL PBS solution containing 0.5 % tween 80 in a shaking bath at 37 °C with a continuous gentle stirring at 100 rpm. 2 mL of the release medium was extracted at given time intervals and at the same time equal volume of fresh medium was appended. The concentration of released PTX was calculated by using the high performance liquid chromatography (HPLC) at specific time intervals. The samples taken for measurement were returned to the receiver solution after measurement. The data were expressed as the mean value of three independent experiments with the standard deviation.

Cell Lines

The cells cultivated for in vitro experiments were human cervix adenocarcinoma (HeLa) cell lines and NIH3T3 (murine fibroblast) obtained from the National Centre for Cell Sciences (NCCS), Pune, India and grown in MEM and DMEM medium, respectively, with 10% fetal calf serum, penicillin ($100U.mL^{-1}$), streptomycin ($100mg.mL^{-1}$), 4×10^{-3} M L-glutamine at 37 °C in a 5% CO2 and 95% air humidified atmosphere.

Nanoparticle-Mediated Cytotoxicity Study

Cells were harvested and the concentration was adjusted to 1×10^{5} $cells.mL^{-1}$. Cells were plated (180mL per well) in a 96 well flat bottom culture plates and incubated with various concentrations of nanoparticles. Cells were incubated with free nanoparticles and PTX-loaded nanoparticles at 37 °C in a humidified incubator, which maintained a constant 5% CO_2. The cytotoxicity was estimated using the MTT assay [3, 4].

Intracellular Uptake Studies

To determine the nanoparticle uptake in HeLa cells, Fe_3O_4@IRMOF-3/FA nanoparticle was labelled with RITC and incubated with cells at a concentration of 5 $mg.mL^{-1}$ for 15 min, 1 h, and 2 h, respectively at 37 °C. After incubation, cells were fixed with 4% paraformaldehyde for 15 min and stained with DAPI ($1mg.mL^{-1}$) for 5 min at 37 °C. Then cells were washed with PBS and examined under fluorescence microscopy (Olympus IX 70) [5, 6].

MR imaging of cells labelled with folic acid modified magnetic NMOFs

A clinical 1.5 T MR scanner (Sigma, GE Medical System, USA) was used to measure Transverse relaxivity (T2) weighted signal intensities (SI). 10^5 numbers of HeLa cells were seeded into a 12-well culture plate before adding the various concentrations of Fe_3O_4@IRMOF-3 and Fe_3O_4@IRMOF-3/FA. The addition of the samples was followed by incubation at 37 °C for 2 h. The repetition time (TR) of 2100 ms and variable echo times (TE) of 100–150 ms were used. Cells were then washed with PBS for three times and fixed with paraformaldehyde. After that, 5 ml of 2% agarose gel was added to each well containing nanoparticle-internalized cells and then allowed to solidify at 4 °C. T2-weighted images were acquired using the following parameters, an acquisition matrix of 256×256, field of view of 240 mm×240 mm, section thickness of 4 mm. The T2 values were calculated by plotting the graph between SI of each sample and TE values.

Data analysis

The statistical analysis was performed by using a statistical package, Origin 6.1, Northampton, MA 01060, USA) with student's *t* tests, $p < 0.05$ as a limit of significance.

Quantification of amine number on the surface of the magnetic NMOFs

To identify the percentage of primary amines in the magnetic NMOFs solution, TNBS assay was used [6]. The nanoscale Fe_3O_4@IRMOF-3 in 4% sodium bicarbonate solution (pH 8.5). 2 mL of nanoscale Fe_3O_4@IRMOF-3 solution was added to 2 mL of 0.1% (w/v) TNBS solution and incubated for 1 h at 40 °C. The magnetic NMOFs were separated from the solution by magnetic decantation. The 1 mL of supernatant was added to 0.1 mL, 40 mmol mL^{-1} of glycine solution. And incubated for 1 h at 40 °C for calculation of unreacted TNBS. Then the reaction was stopped by adding 1 mL of 2 N HCl, and the sample was consequently hydrolyzed

for 20 min at the same temperature. The absorbance value was read at 410 nm using Shimadzu UV-1700 spectrophotometer. The $–NH_2$ group concentration was calculated by comparing the value with the standard curve originated by taking glycine (0–2 mmol) as standard.

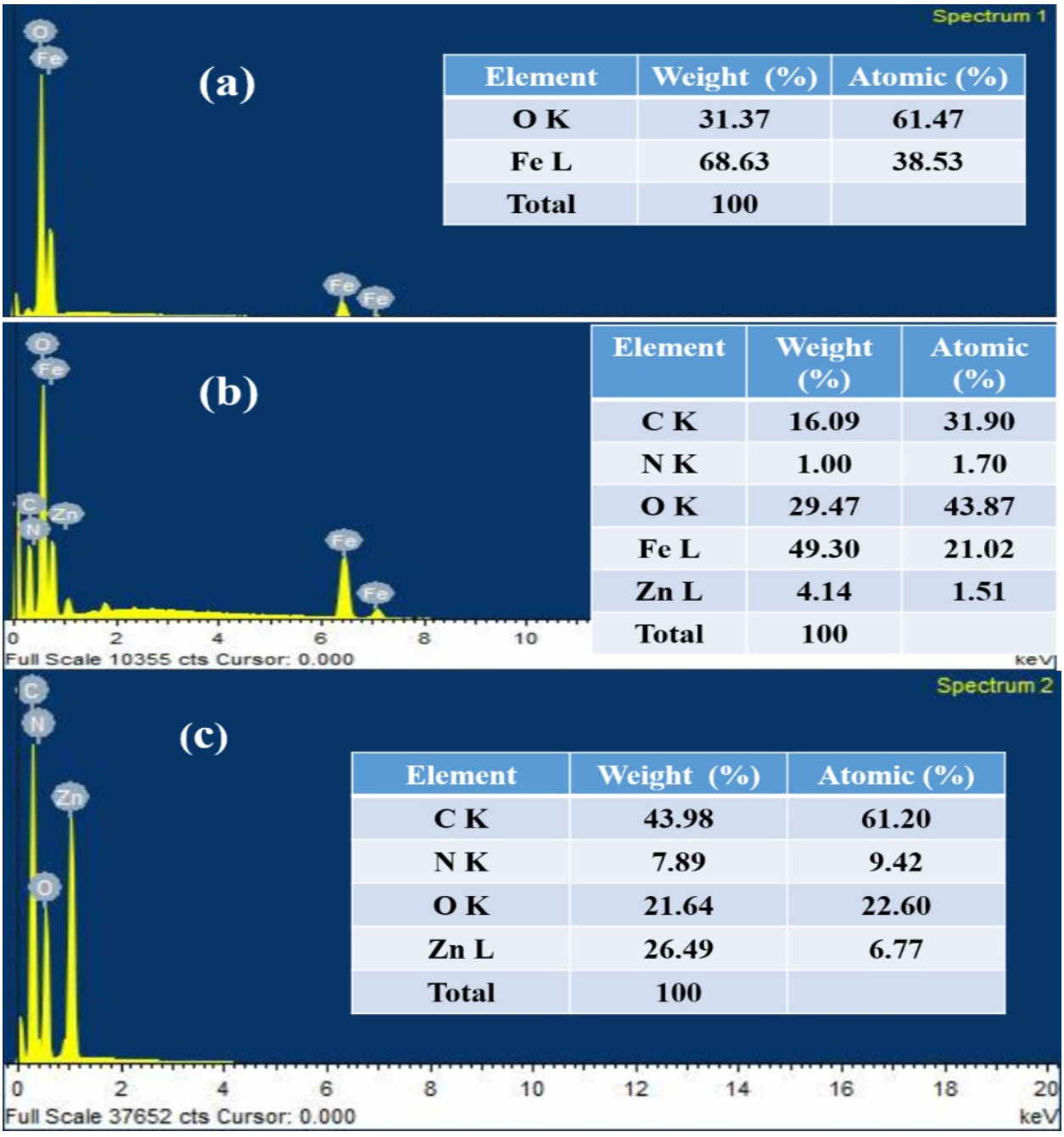

Figure S1: EDAX spectrum of (a) Only Fe_3O_4 NPs (b) nanoscale magnetic Fe_3O_4@IRMOF-3 and (c) nanoscale IRMOF-3.

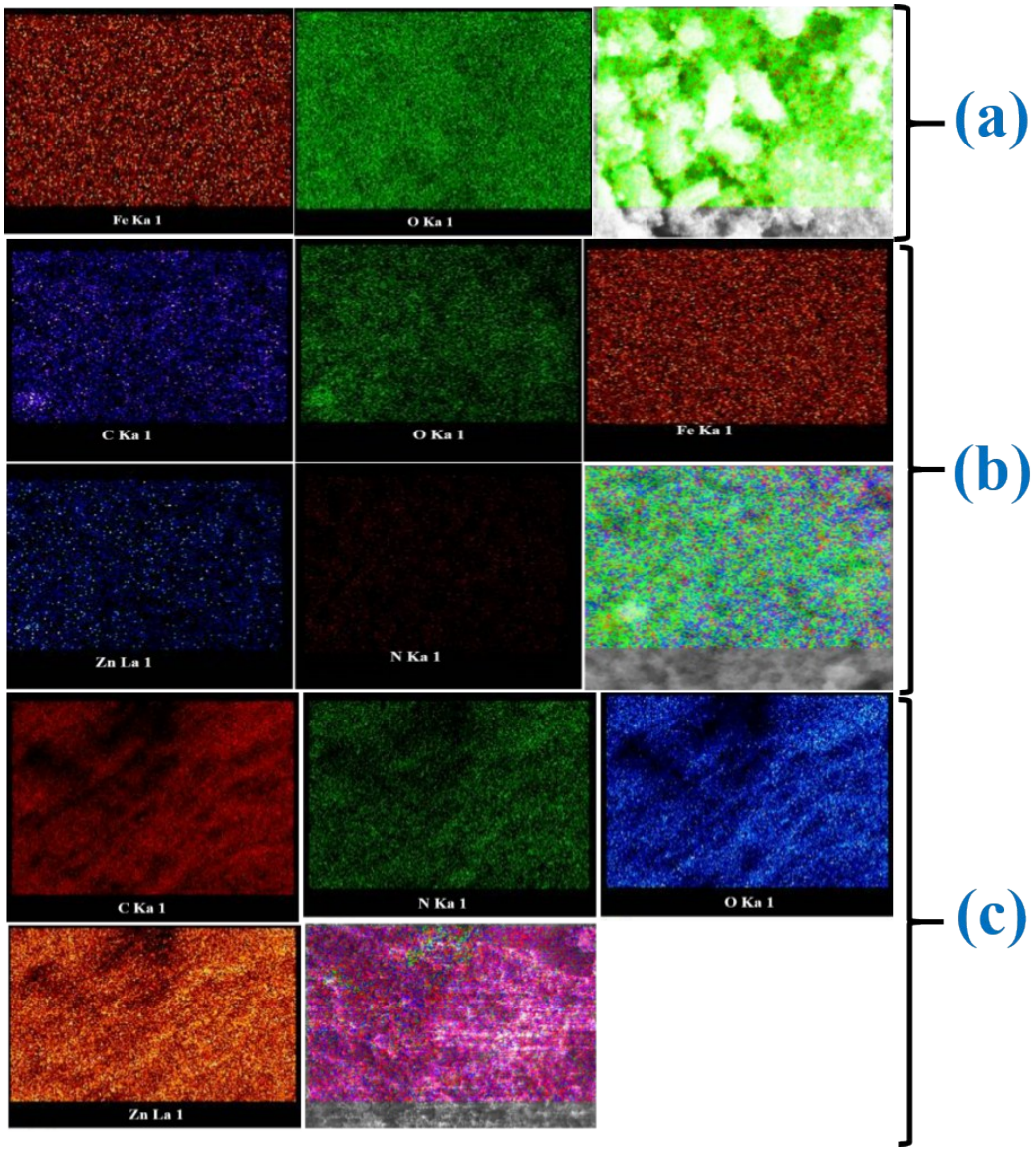

Figure S2: Elemental mapping of (a) Fe_3O_4 NPs (b) nanoscale magnetic Fe_3O_4@IRMOF-3 and (c) nanoscale IRMOF-3.

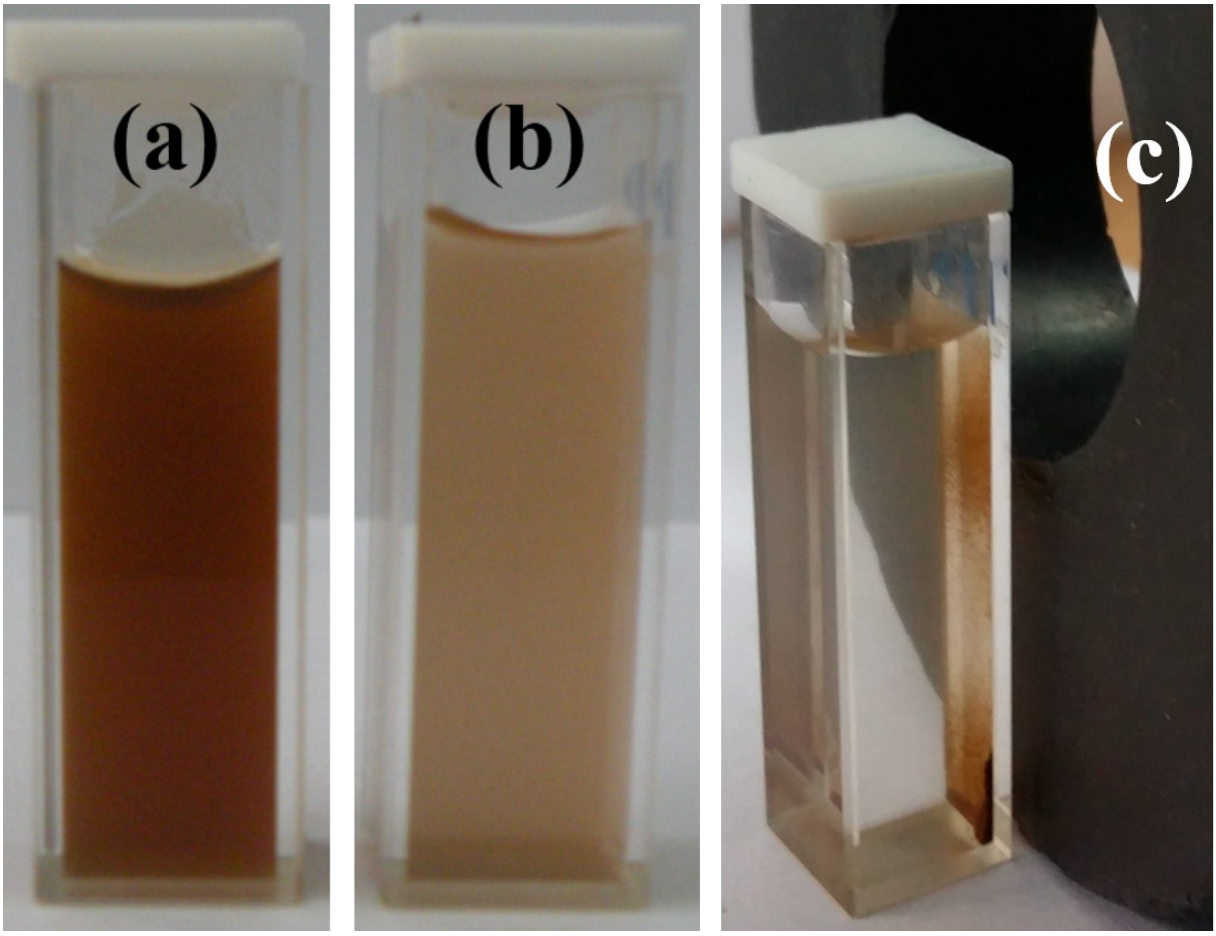

Figure S3: Camera photo image of (a) magnetic nanoscale Fe_3O_4@IRMOF-3 (b) folic acid targated magnetic nanoscale Fe_3O_4@IRMOF-3 and (c) magnetic separation of folic acid targeted magnetic nanoscale MOFs.

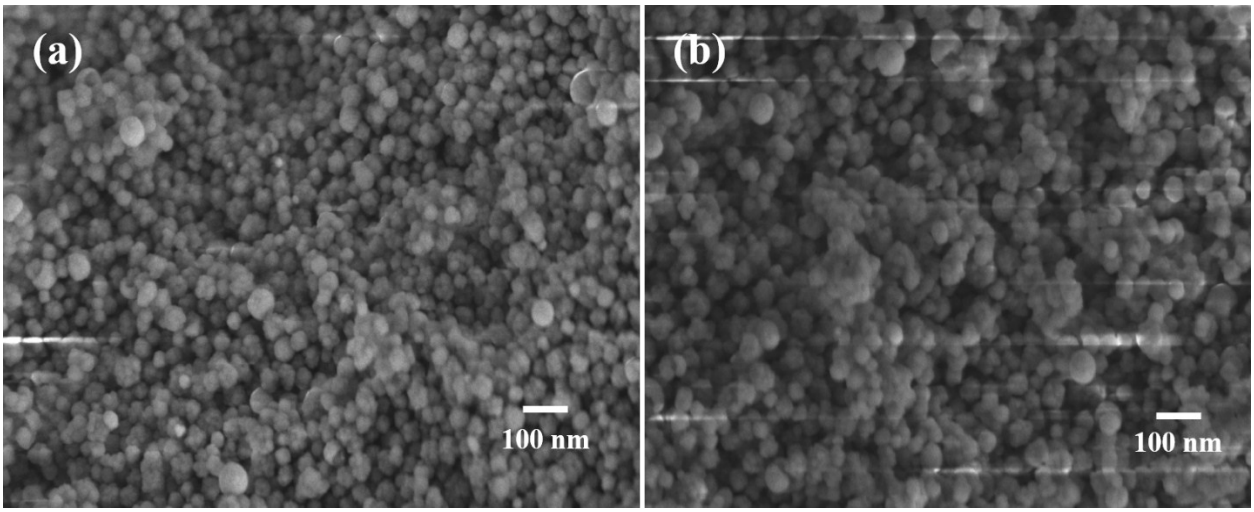

Fig. S4. FESEM image of synthesized magnetic nanoscale Fe_3O_4@IRMOF-3/FA after stirring 12h (a) and 24 h (b) in PBS at 37 ^oC.

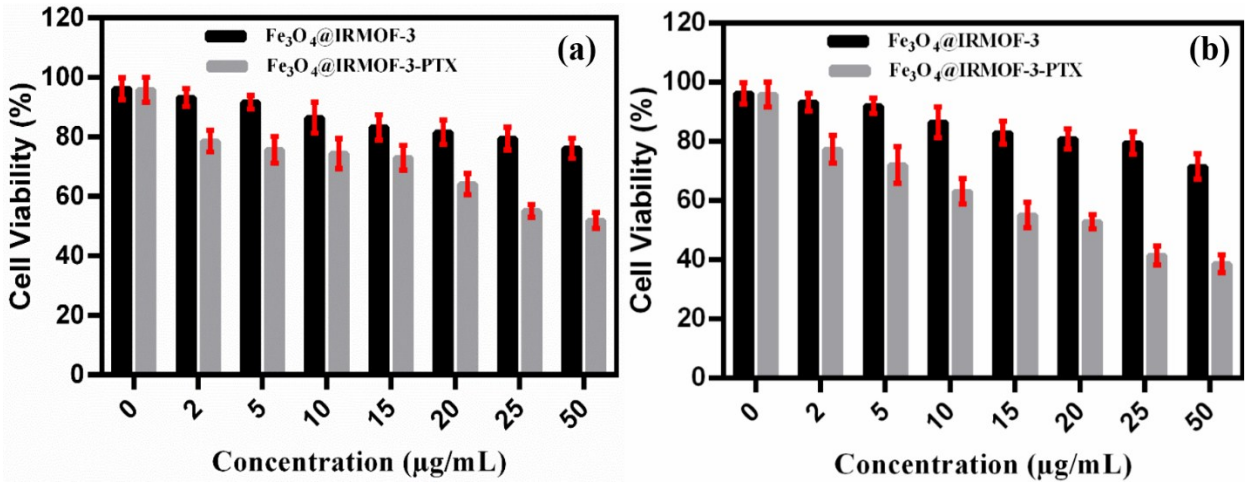

Fig. S5. Cell viability of (a) NIH3T3 cells (b) HeLa cells treated with Fe_3O_4@IRMOF-3 NMOFs and PTX loaded Fe_3O_4@IRMOF-3 NMOFs at different concentrations. Cells were incubated with NPs for 24 h.

References

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