Supplementary Information

Fe_3O_4@Zirconium Phosphate Core-shell Nanoparticles for pH-Sensitive and Magnetically Guided Drug Delivery Application

Himani Kalita[a], Shashi Rajput[b], B. N. Prashanth Kumar[b], Mahitosh Mandal[b], Amita Pathak[a,*]

[a] Department of Chemistry, Indian Institute of Technology Kharagpur, West Bengal-721302, India

[b] School of Medical Science and Technology, Indian Institute of Technology Kharagpur, West Bengal-721302, India

* Corresponding author:

**E-mail address*: ami@chem.iitkgp.ernet.in

*Tel.: +91-3222-283312, +91 94340 38730; Fax: +91-3222-282252

1. Detection of ROS generation

The intracellular generation of reactive oxygen species (ROS) from the prepared nanoparticles (viz. magnetite, FeZP0.07, and FeZP0.04) was measured using the oxidation sensitive dye 5-(and -6) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA). For the study, MDA-MB-231 cell lines were treated with the nanoparticles for 24 h, after which the cells were trypsinized and suspended in PBS with CM-H_2DCFDA (10 µmol/L) for 1 h and subsequently washed with PBS to remove the unreacted dye. The samples were then acquired on the FL-1 channel of FACSCalibur (BD, Bioscience, USA) and analyzed using Cellquest software. For the analysis, untreated MDA-MB-231 cell lines were taken as the control. The generation of ROS from the nanoparticles was evaluated from the fluorescence measurement of the cell lines and the results are presented in Fig. S1. A dramatic ROS burst was observed in the cells treated with MNPs in contrast to the control, with profound peak shift annotating the intense fluorescence. On the contrary, insignificant peak shift was observed in the cells treated with FeZP0.04 inferring the production of ROS by the nanoparticles to be comparable with that in control cells. Further, FeZP0.07 treatments showed a slight shift of peak that indicates a small production of ROS by the nanoparticles in contrast to control cells. Thus, it can be inferred that MNPs elicits ROS production, which gets significantly reduced with the increase in thickness of zirconium phosphate shell on magnetite core in the core-shell nanoparticles.

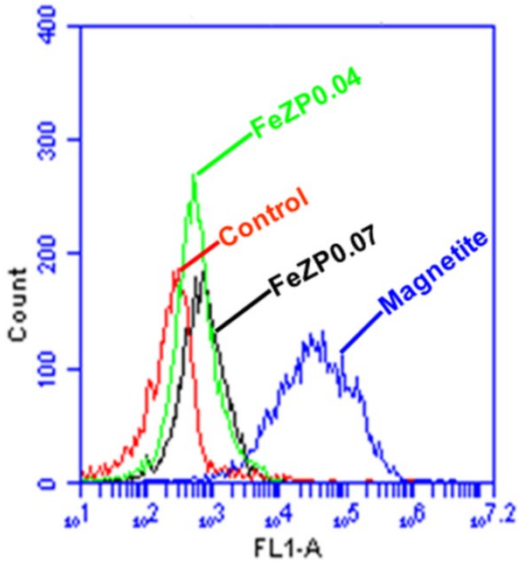

Fig. S1 Quantitative measurement of ROS in MDA-MB-231 cells after 24 h of treatment with the nanoparticles.

2. DPPH radical scavenging analysis

The generation of ROS from the nanoparticles (i.e. magnetite, FeZP0.07, and FeZP0.04) was further ascertained from their DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The purple colored DPPH molecule contains a stable free radical that changes to a yellow colored stable compound upon reaction with a ROS. The decaying of purple coloration of the DPPH solution, that can be monitored spectrophotometrically at 517 nm thus, will give a measure of the ROS produced by the nanoparticles. For the study, 10 mg nanopaticles were added to 2 mL of 20 μM DPPH solution in ethanol and agitated for 30 min in absence of light. Subsequently, the absorbance of the supernants was measured at 517 nm and the scavenging percentage of the samples were calculated using the following formula:

DPPH scavanging (%) = $(A_C\text{-}A_S/A_C) \times 100$

where, A_C is the absorbance of blank DPPH and A_S is the absorbance of DPPH solution in presence of nanoparticles. The results depicted in Fig. S2 revealed that MNPs exhibit DPPH radical scavenging of 71.1%, whereas the scavenging was observed to be 36.2 and 13.3% in FeZP0.07 and FeZP0.04 respectively. The decrease in DPPH scavenging from MNPs to FeZP0.04 indicated a significant reduction in ROS generation with increase in the thickness of zirconium phosphate shell on magnetite core.

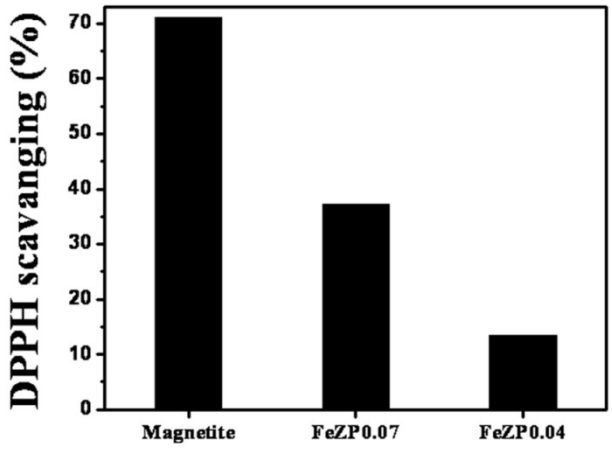

Fig. S2 DPPH radical scavenging percentage of the nanoparticles.

3. Hemocompatibility assay

Hemocompatibility and hemolysis are major attributes for assessment of therapeutic intervention of nanoparticles in drug delivery application, where nanoparticles with hemolysis of

5% or less are considered as non toxic and biocompatible[1] in therapeutic perspective. The hemocompatibility of the prepared nanoparticles (i.e. magnetite, FeZP0.07, and FeZP0.04) was ascertained from their hemocompatiblity assay. The red blood cells (RBC) were collected from the blood of BALB/c male mice (6 weeks older) by centrifugation using a ficoll density gradient. For the assay, the collected RBC pellets were diluted in PBS (5% v/v) and then added to PBS (-ve control), 1% Triton X-100 (+ve control) and the nanoparticles (suspended in 1X PBS), which were then incubated at 37 °C for 30 and 60 min. The suspensions were centrifuged and the absorbance of the supernatants was measured at 570 nm. The results of hemolytic assay (shown in Fig. S3) revealed 15 and 23% hemolysis by MNPs after 30 and 60 min respectively. On the contrary, insignificant percent of hemolysis (less than 5%) was observed in FeZP0.07 and FeZP0.04 treatments even after extended exposure till 60 min, where the hemolysis was lowest for the FeZP0.04 nanoparticles. Hemolytic assay of the nanoparticles indicates that the FeZP0.04 nanoparticles are hemocompatible and suitable for potential usage in biomedical fields.

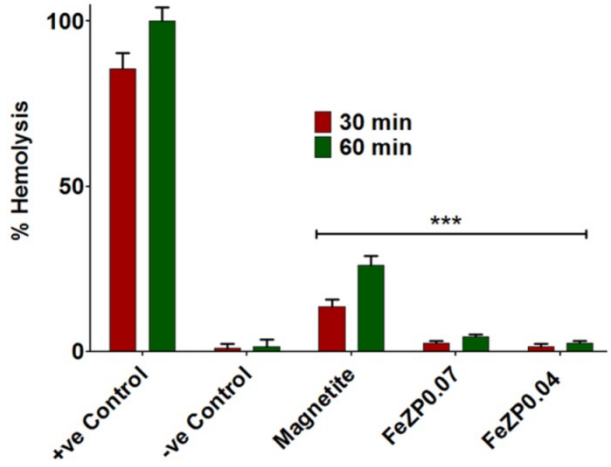

Fig. S3 Hemolytic assay of magnetite, FeZP0.07, and FeZP0.04 nanoparticles, where PBS and 1% Triton X-100 was taken as Negative and Positive controls respectively. Each bar indicate the means ± SD (n = 3) with $P < 0.001$.

4. References

1 M. He, Z. Zhao, L. Yin, C. Tang and C. Yin, *Int. J. Pharm.*, 2009, **373**, 165-173.