Supporting Information

Folic acid-conjugated magnetic mesoporous silica nanoparticles loaded with quercetin: A theranostic approach for cancer management

Snehasis Mishra[1,5], Krishnendu Manna[1], Utpal Kayal[2], Moumita Saha[1], Sauvik Chatterjee[2], Debraj Chandra[3], Michikazu Hara[4], Sriparna Datta[5], Asim Bhaumik,[2,*] Krishna Das Saha[1,*]

[1]Cancer Biology and Inflammatory Disorder Division, CSIR-Indian Institute of Chemical Biology, Kolkata-700032, West Bengal, India

[2]School of Material Science, Indian Association of Cultivation of Science, Kolkata-700032, West Bengal, India

[3]World Research Hub Initiative (WRHI), Institute of Innovative Research, Tokyo Institute of Technology, Nagatsuta-cho 4259, Midori-ku, Yokohama 226-8503, Japan

[4]Laboratory for Materials and Structures, Institute of Innovative Research, Tokyo Institute of Technology, Nagatsuta-cho 4259, Midori-ku, Yokohama 226-8503, Japan

[5]Department of Chemical Technology, University of Calcutta, Kolkata-700009, West Bengal, India

***Corresponding Authors:** E-mail: msab@iacs.res.in (AB); krishna@iicb.res.in (KDS)

Assessment of cell viability

Cell viability was checked for determining the cytotoxic effect of mesoporous silica nanoparticles (SBA-15), folic acid (FA), quercetin (QN), iron oxide (Fe_3O_4), folic acid conjugated quercetin loaded in SBA-15 (FA-SBA15QN), and folic acid conjugated magnetic mesoporous silica nanoparticle conjugated with quercetin (FA-FE-SBA15QN) by MTT [(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] assay. Cells at a required density (3×10^3 cells/well) were seeded in 96 well plates and treated with different concentrations of

SBA-15, FA, QN, Fe_3O_4, FA-SBA15QN, and FA-FE-SBA15QN. Quickly after treatment, plates were kept in an incubator at 37 °C in a humidified CO_2-rich condition (5%). After completion of the incubation period, cells were washed with PBS followed by the addition of MTT solution (4 mg/mL) and kept it in an incubator for 4 h. The absorbance of the DMSO-solubilized intracellular formazan salt was documented at 595 nm using an ELISA reader (EMax, Molecular Device, USA). In all cases, the samples were sonicated before treating in a cell line to get homogenized mixtures. Another set of cell viability assessment was done with IC_{50} of native QN and FA-FE-SBA15QN along with/without N-acetylcysteine, JNK inhibitor (SP600125), siRNA specific for p53/HSP27.

Biodistribution of nanoparticles by ICP-MS

A single set of experimentation on tumor bearing mice was carried out to investigate the bioavailability of SBA-15, iron oxide nanoparticle (FE-NP) and FA-FE-SBA-15. Mice were treated intraperitoneally with 15 mg/kg of FA-FE-SBA15QN, Fe_3O_4-NP and SBA-15, followed by the inductively coupled plasma mass spectrometric analysis (ICP-MS) to observe the biodistribution in liver and tumor tissue. After the completion of 1st and 6th treatment schedule (for six alternative days), organs were collected and lysed in the digestion solution containing Perchloric acid ($HClO_4$) and nitric acid (HNO_3) of 1:5 ratio according to the standard protocol. Until the reaction equilibrium was achieved, the solution was heated to 230°C–280°C. The digested tissue samples were used to determine the concentrations of nanoparticles by ICP-MS which was expressed as ng/mg of tissue.

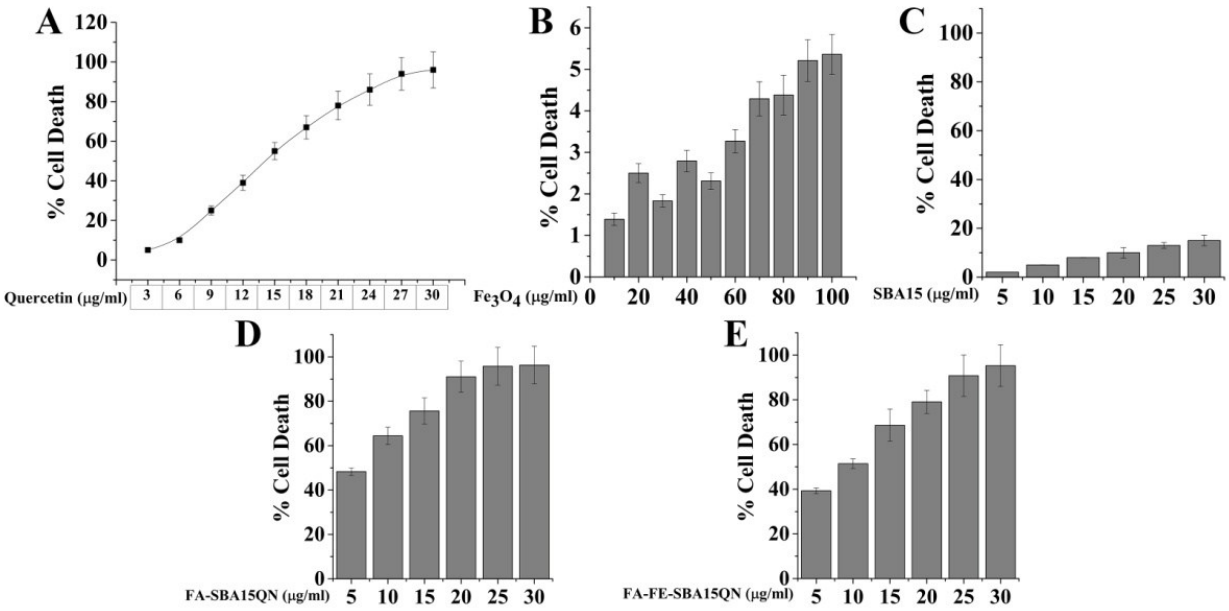

Fig. S1. Assessment of cell viability upon the treatment of different concentration of QN, Fe_3O_4, SBA-15, FA-SBA-15QN, and FA-FE-SBA15QN. Values are represented as mean ± SEM (n=5).

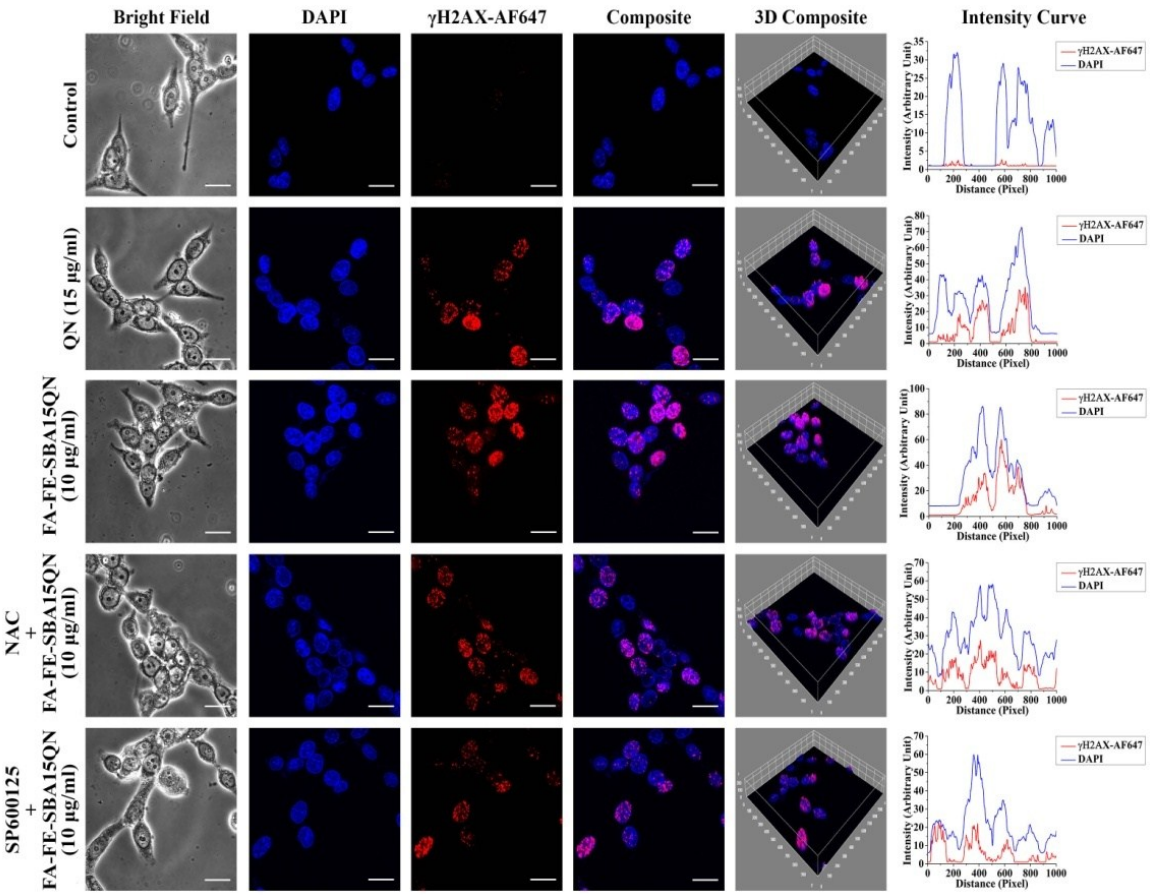

Fig. S2. QN and FA-FE-SBA15QN-induced DSBs were marked by γH2AX foci. Immunofluorescence images showing the expression of γH2AX. DAPI was used for nuclear staining. Slides were viewed using a confocal microscope (Magnification 20×). Respective fluorescence intensities (γH2AX-AF647 and DAPI) were analyzed using ImageJ software through RGB calculator.

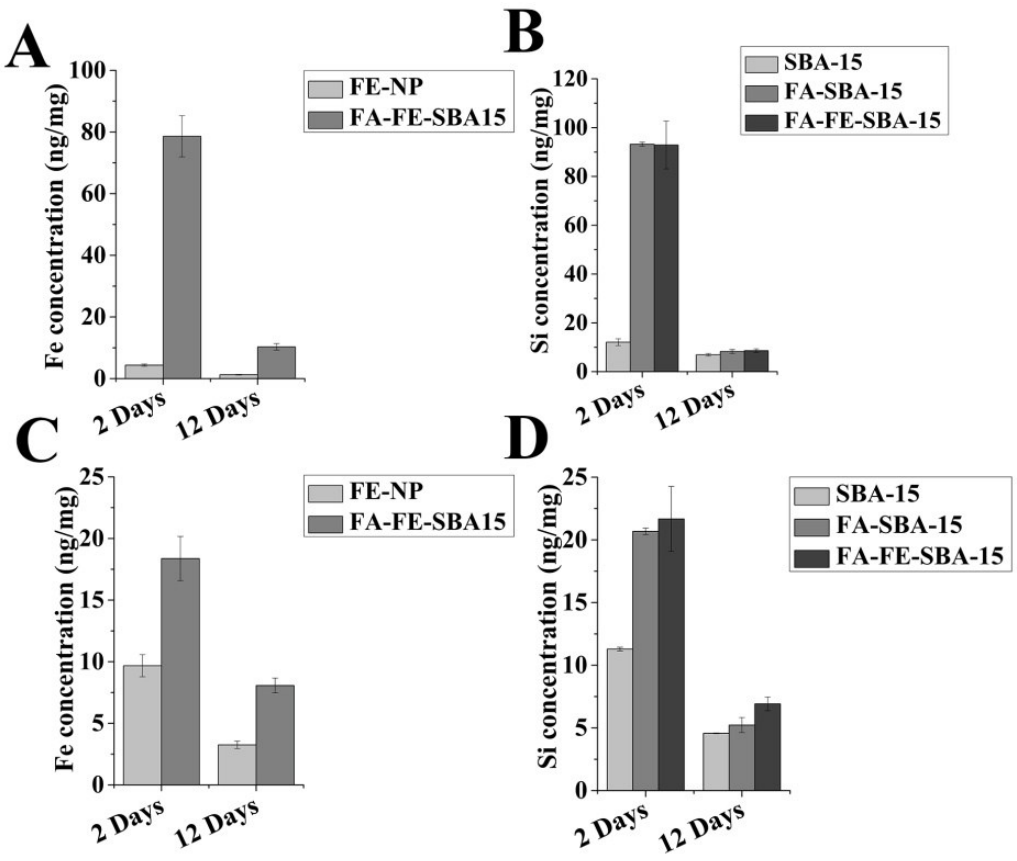

Fig. S3. The concentration of iron and silica content of IO-NP, SBA-15, FA-SBA-15FA-FE-SBA15QN in (A and B) tumor tissue, (C and D) Hepatic tissue after the single application of respective nanoparticles in 2nd day and 12th day. Values are represented as mean ± SEM (n=6).