Electronic Supplementary Material (ESI) for RSC Advances.

Magnetoelectric core-shell $CoFe_2O_4$ @ $BaTiO_3$ nanorods, their role in drug delivery and effect on multidrug resistance pump activity *in vitro*

Sadaf Mushtaq[1,2], Khuram Shahzad[3], Muhammad Rizwan[3], Anwar Ul-Hamid[4], Bilal Haider Abbasi[1], Waqas Khalid[3], Muhammad Atif[3], Nafees Ahmad[2], Zulqurnain Ali[3], Rashda Abbasi[2*]

[1]Department of Biotechnology, Quaid-i-Azam University Islamabad, Pakistan

[2]Institute of Biomedical and Genetic Engineering, G-9/1, Islamabad, Pakistan

[3]Functional Materials Lab, Department of Physics, Air University Sector E-9, Islamabad Pakistan

[4]Core Research Facilities, King Fahd University of Petroleum & Minerals, Dhahran 31261, Saudi Arabia

Supplementary Information

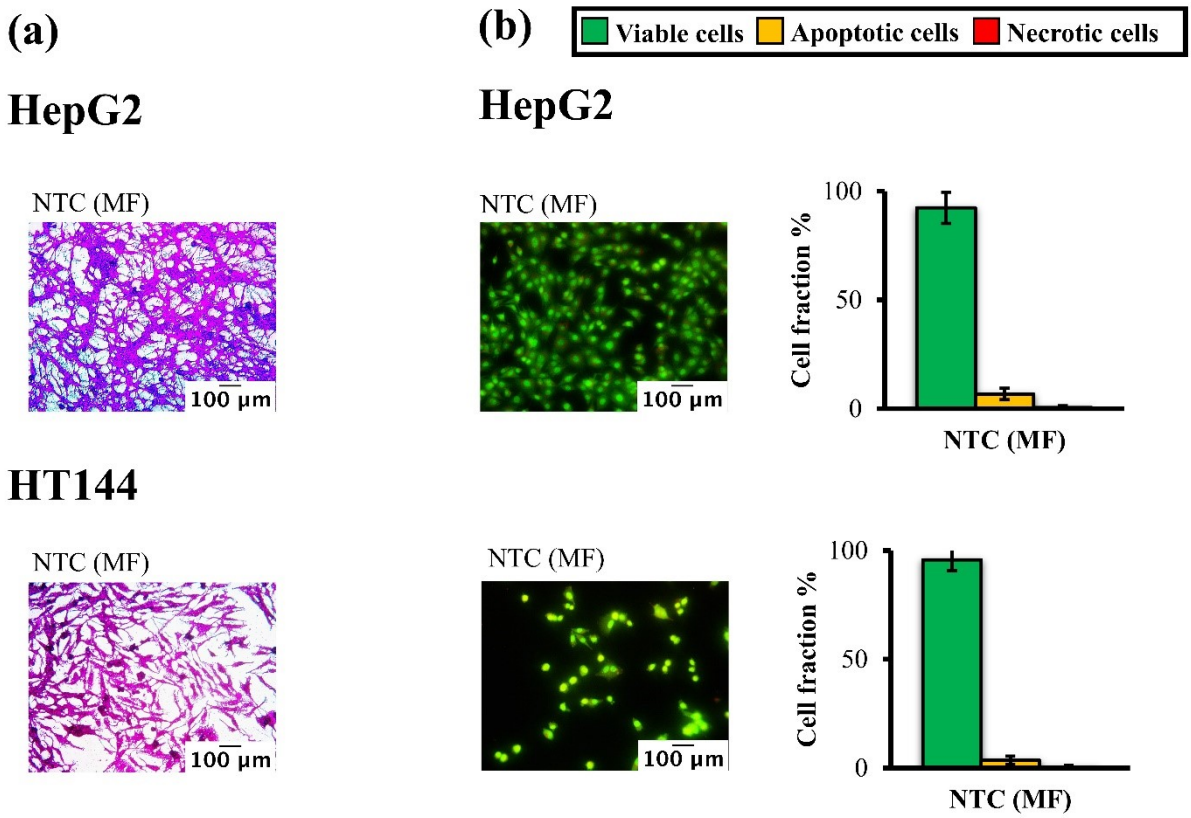
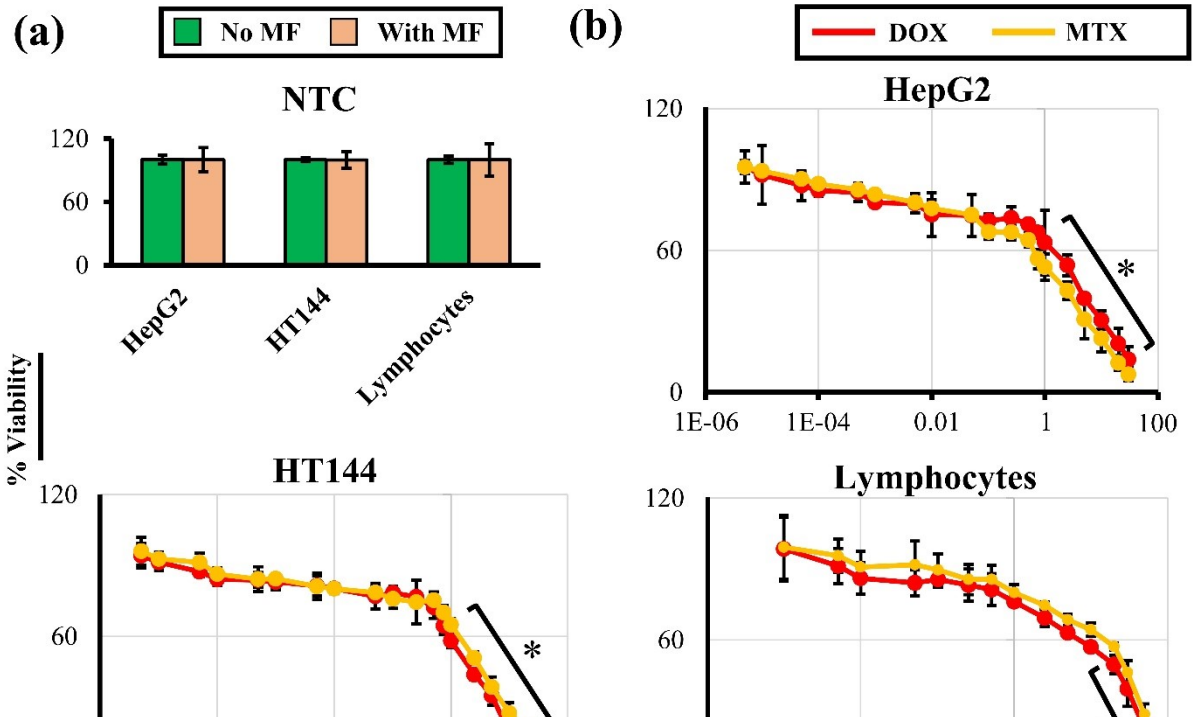


Figure S1: (a) Cytotoxicity screening (SRB) and **(b)** Fluorescent microscopic images (Acridine orange and Propidium Iodide staining: AOPI) of untreated (NTC) HepG2 and HT144 cells (Magnification = 200X, scale bar = 100 µm) in the presence of external magnetic field (MF = 4 mT; 20 min). Bar charts indicate percent fractions of live, necrotic, and apoptotic cells after AOPI staining. Data is represented as mean ± SD of experimental triplicates.

Figure S2: **(a)** Percent viability of untreated (NTC) HepG2, HT144 and lymphocytes cells in the presence and absence of external MF (4 mT, 20 min) after 24 hours. **(b)** Dose response curves of DOX and MTX in HepG2, HT144 and freshly isolated human lymphocytes. HepG2 & HT144 cells were treated with (0.000005 - 30 µM) and lymphocytes with (0.001 – 100 µM) concentrations of DOX and MTX for 24 hours. Data plotted represents mean ± SD of experimental replicates. Doses on x-axis are plotted as log values. *p<0.05 (two-tailed t test when compared to untreated cells). Non-linear regression curve analysis (GraphPad Prism 9.4.0) was performed to determine IC_{20}, IC_{50}, and IC_{80} concentrations (µM).



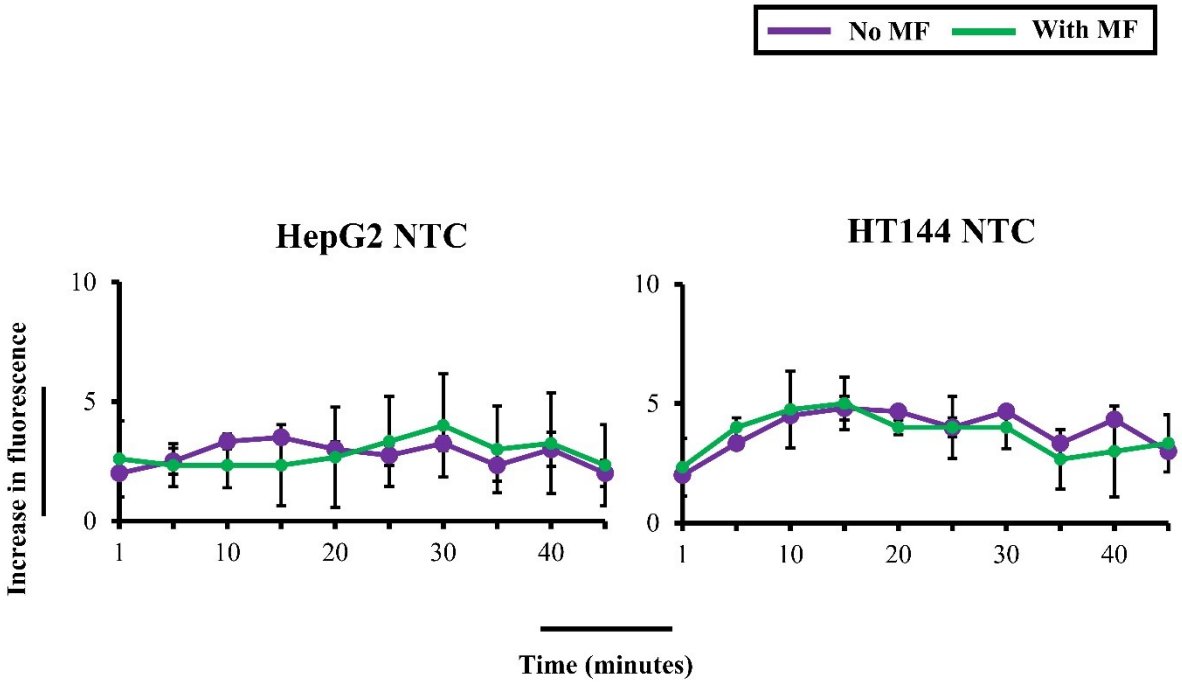
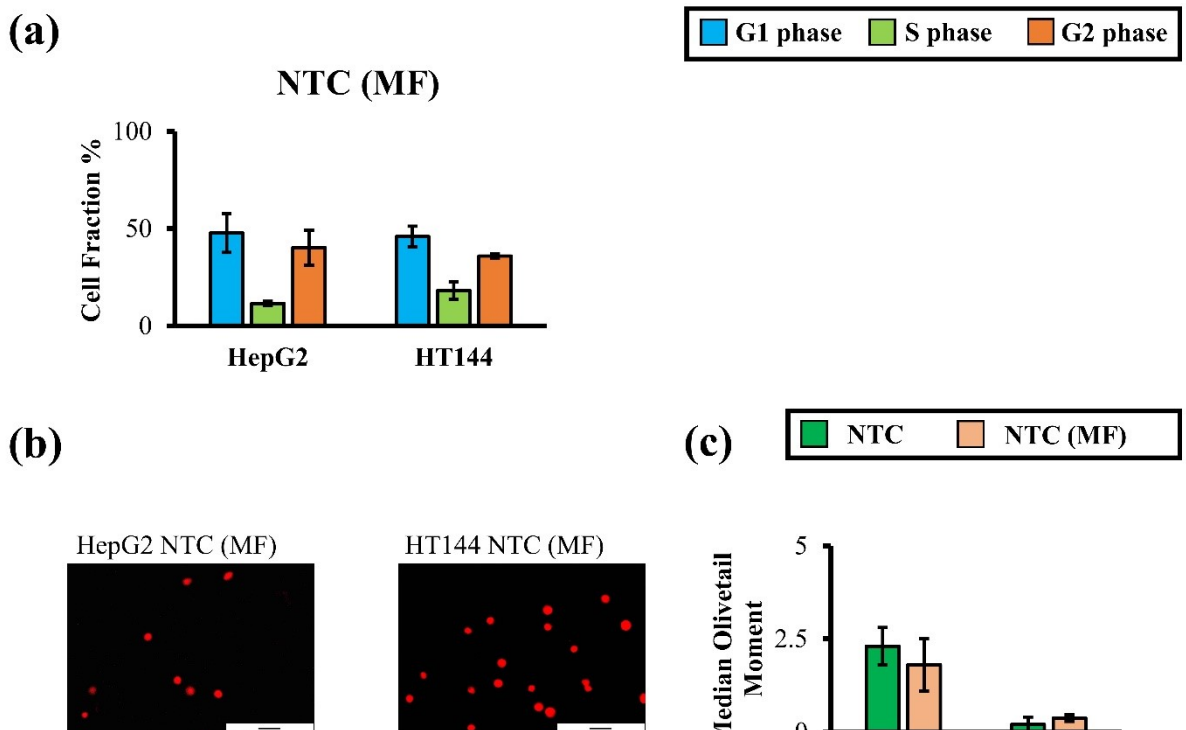


Figure S3: Measurement of time dependent (1 - 45 minutes) intracellular oxidative stress in untreated HepG2 and HT144 cells in the presence and absence of external MF (4 mT, 20 minutes). Increase in fluorescence indicate extent of ROS production. These values were used for relative calculations in figure 5(b). Data is represented as mean ± SD of experimental triplicates.

Figure S4: (a) Flow cytometric analysis of cell cycle progression in untreated (NTC) HepG2 and HT144 cells under the influence of MF (4 mT, 20 minutes) after 24 hours. **(b)** Fluorescent microscopic images (Magnification = 200X, Scale bar = 100 µm) of untreated (NTC) HepG2 and HT144 cells in the presence and absence of MF (4 mT, 20 minutes) after 1 hour. **(c)** Bar chart indicating measurement of genotoxicity in the form of median olive tail moments. These measurements were used for relative calculations in figure 6(c). Data represents mean ± SD of three experimental replicates for both assays.



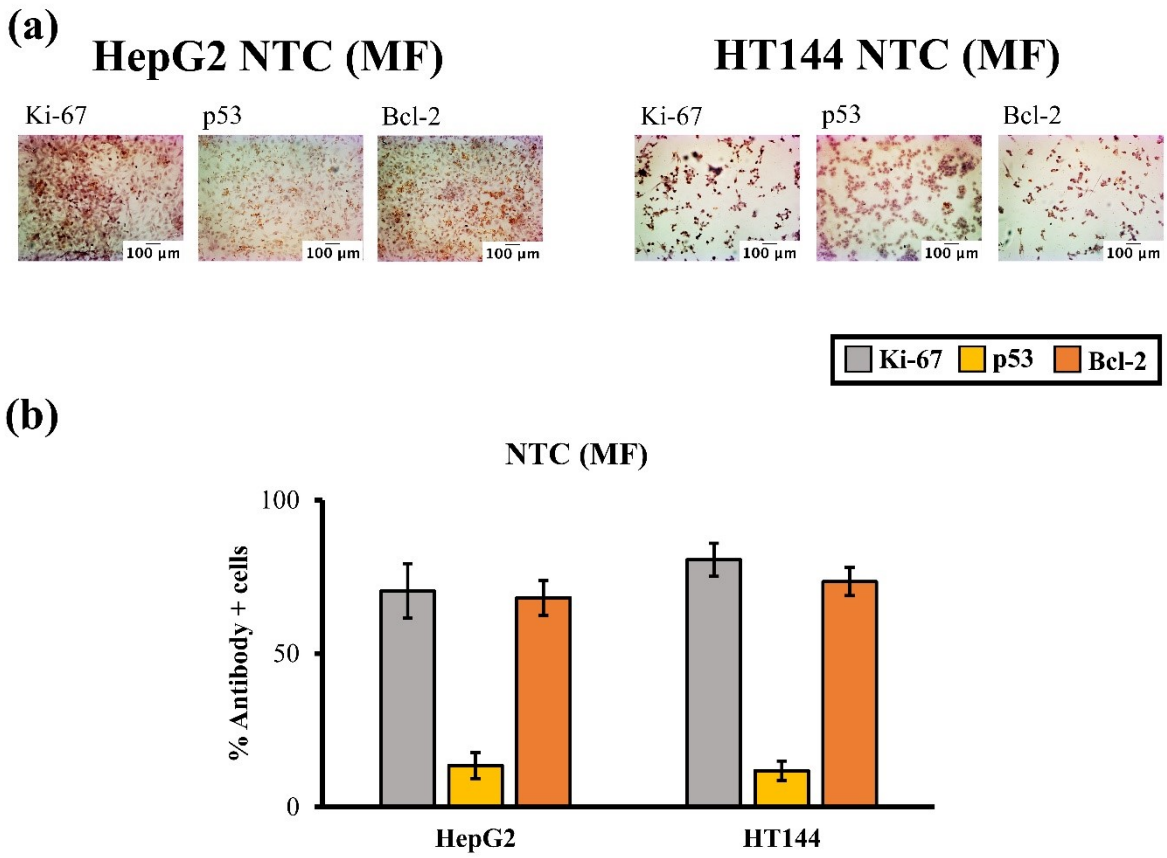


Figure S5: (a) Microscopic images (Magnification = 200X, Scale bar = 100 µm) of immunocytochemical (ICC) assessment of Ki-67, p53 and Bcl-2 cancer biomarkers in untreated HepG2 and HT144 cells under MF influence (4 mT, 20 minutes) after 24 hours. **(b):** Quantitative analysis of ICC (HepG2 and HT144: NTC (MF)) with antibody positive cells counted and plotted as percentages (mean ± SD).

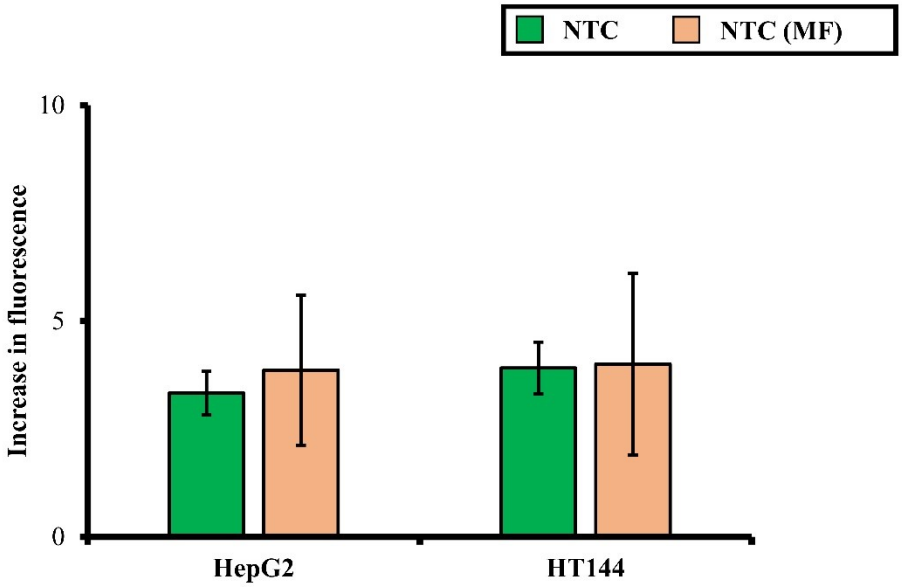


Figure S6: Multidrug resistance (MDR) pump activity in untreated HepG2 and HT144 cells with and without MF assistance (4 mT, 20 minutes) after 24 hours incubation. Increase in fluorescence indicate increase in MDR pump inhibition. These values were used for relative calculations in figure 8. Data represented as (mean ± SD) of experimental triplicates.

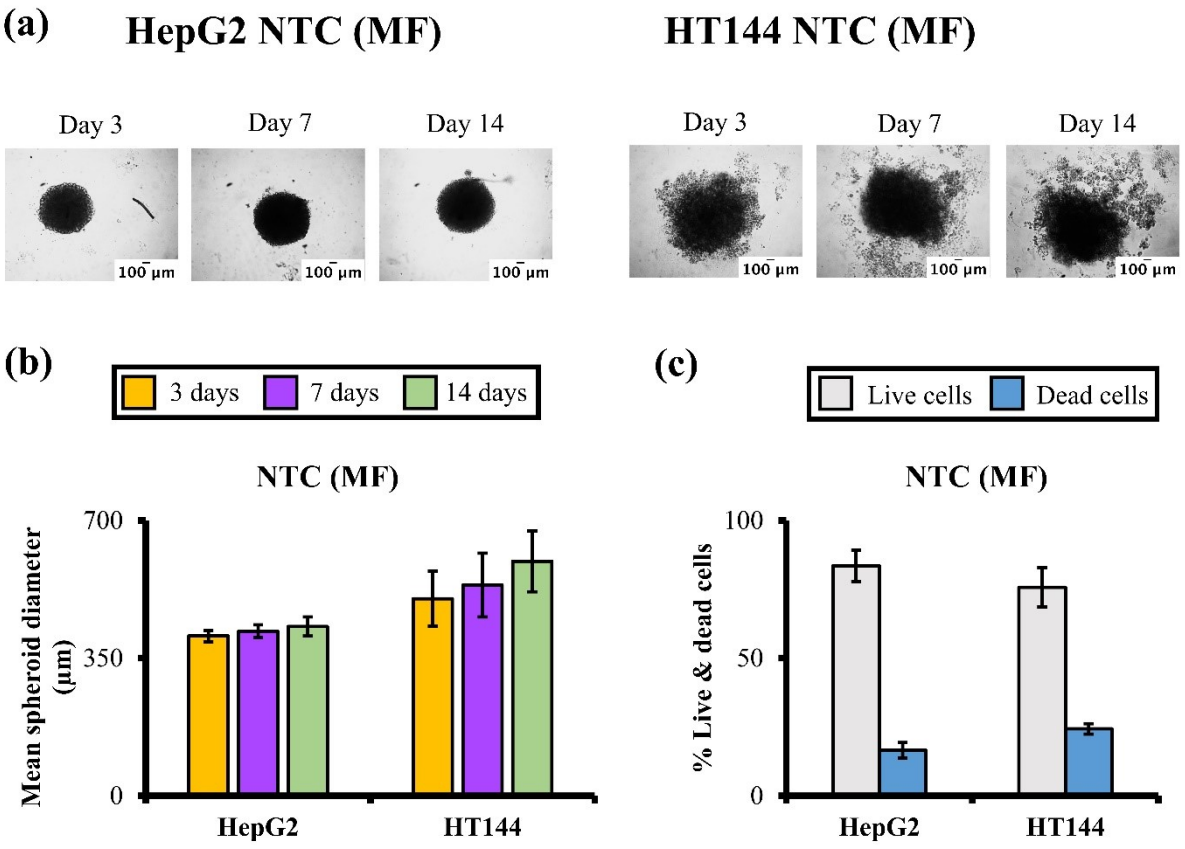


Figure S7: (a) Microscopic images representing effect of MF (4mT, 20 minutes) on untreated HepG2 and HT144 3D spheroids after 3, 7 and 14 days. Magnification = 100X, Scale bar = 100 µm. **(b)** Bar charts indicating average changes in untreated HepG2 and HT144 spheroids diameter in the presence of MF (4 mT, 20 minutes) after 3, 7 and 14 days. Mean ± SD spheroid diameters were calculated using ImageJ software at each time point. Multiple regions were covered to include all maximum and minimum diameter ranges of spheroids. **(c)** Determination of cellular viability via trypan blue assay at 14^{th} day in untreated HepG2 and HT144 spheroids in the presence of MF (4 mT, 20 minutes). Data presents mean ± SD of three replicates.

Table S1: IC_{20}, IC_{50} and IC_{80} concentrations of free DOX and MTX in HepG2, HT144 and freshly isolated human lymphocytes.

Cell type	**DOX (µM)**			**MTX (µM)**		
	IC_{20}	**IC_{50}**	**IC_{80}**	**IC_{20}**	**IC_{50}**	**IC_{80}**
HepG2	0.16	2.52	21.04	0.11	1.35	9.15
HT144	0.18	1.82	8.74	0.24	2.47	17.08
Lymphocytes	1.71	14.81	56.84	3.6	21.78	67.5