Supporting Information

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Design and synthesis of gatekeeper coated dendritic silica/titania mesoporous nanoparticles with sustained and controlled drug release properties for targeted synergetic chemo-sonodynamic therapy

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2. EXPERIMENTAL

2.1. Materials

Tetraethyl orthosilicate (TEOS), cetylpyridinium bromide (CPB), folic acid (≥97%), N, N-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (NHS), polyethylenimine (MW = 10 kD), paraformaldehyde, and curcumin were purchased from Sigma-Aldrich Co. 4′,6-Diamidino-2-phenylindole (DAPI**),** tetrabutyl titanate **(**$Ti(OBu)_4$), cyclohexane, n-butanol, ammonium nitrate, and acetonitrile were provided from Merck.

2.2. Characterizations

IR, ^{1}HNMR, and UV–Vis spectra were recorded on Bruker Vector 22 FT-IR, Bruker 500Avance, and III JASCO-570, respectively. XRD patterns were recorded on a Philips X-ray diffractometer using Ni-filtered Cu$K\alpha$ radiation in the 2θ range of 10-100º. The morphology of the prepared nanoparticles was determined by field-emission scanning electron microscopy (FE-SEM) by a JEOL scanning electron microscope. Transmission electron microscopy (TEM) images were obtained using a Zeiss-EM10C-80 kV (Germany). RP-HPLC analysis was carried out on an Agilent system (USA) with an analytical C18 column. The mobile phase consisted of acetonitrile and distilled water (40/60, v/v) with a flow rate of 1 mL/min. The injection volume was 20 µL and the detection wavelength was 425 nm. Nitrogen adsorption-desorption isotherms were measured at –196 °C using a Japan BELSORP-II system after the samples were vacuum dried at 150 °C overnight. Zetasizer Nano ZS (Malvern Instruments, United Kingdom) equipped with a laser (633 nm) was used to determine the evolution of the hydrodynamic diameter and surface charge of the prepared nanoparticles by dynamic light scattering (DLS) and zeta (ζ)-potential measurements, respectively.

The thermal features of the samples were determined by a thermogravimetric analyzer (TGA7, PerkinElmer) under N_2 atmosphere at 10 ^oC min^{-1} heating rate. The cell images were recorded by an Olympus IX81 fluorescence microscope that equipped with an Olympus DP_{70} camera (Olympus Corp., Tokyo, Japan).

2.8. Cell culture

The Hela and A549 cancer cell lines were provided from the Culture Collection of the Shiraz University of Medical Sciences in Iran. The cells were incubated in a humidified incubator in standard conditions (37 °C and 5% CO_2 atmosphere) and grown in a RPMI 1640 medium including 10% (v/v) heat-inactivated fetal bovine serum (Gibco), penicillin and streptomycin (both at 1% w/v) and glutamine (2 mM).

2.9. Cell viability assay

The *in vitro* cytotoxicity of the free CUR, free FA-PEI-DSTNs, and CUR@FA-PEI-DSTNs system under various US irradiation times was investigated by the MTT cytotoxicity assay. The cells were seeded in 96-well plates (5×10^3 cells per well) and grown at 37 °C in a humidified 5% CO_2 incubator. In the next step, the free CUR, free FA-PEI-DSTNs, and CUR@FA-PEI-DSTNs system (all in DMSO) were added to the wells to make final concentrations ranging from 1 to 200 μg/mL. After the incubation at 37 °C for 48 h, the stock of the MTT dye solution was added to each well. Followed by a 4 h incubation, the MTT containing medium was replaced by 200 μL of DMSO. The 96-well plates were then oscillated for 10 min to completely dissolve the purple formazan crystals formed by the living cells in the wells. The absorbance was monitored at 570 nm (for the measurement) and 630 nm (for the reference) by a microplate reader.

The cytotoxicity of the free CUR, free FA-PEI-DSTNs, and CUR@FA-PEI-DSTNs system without and with a low-intensity ultrasound (1 MHz, 2 W/cm^2) for 30, 60, and 120 s was estimated as the percentage ratio of the absorbance of the treated cells to the untreated cells.

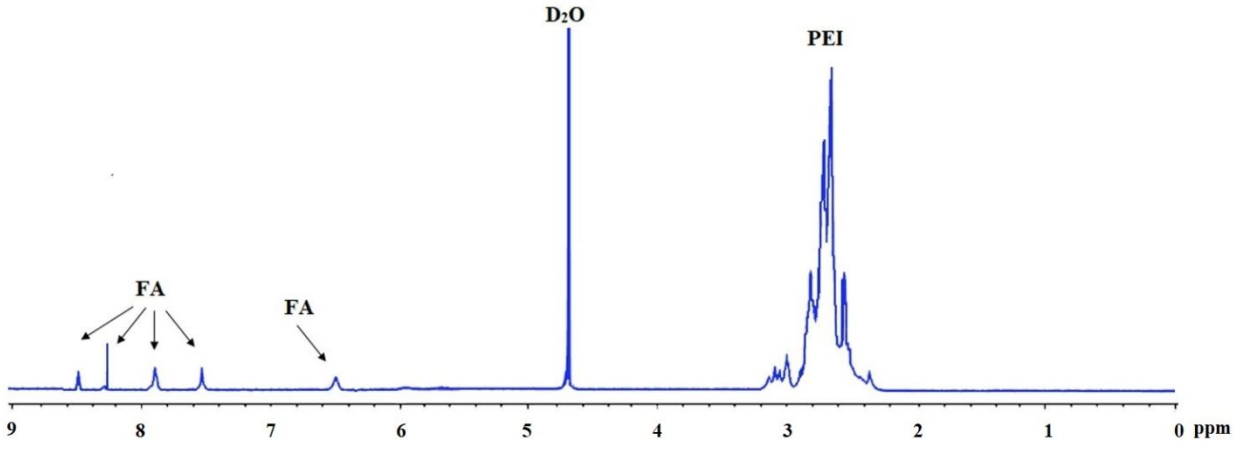

Figure S1. ^{1}HNMR spectrum of PEI-FA in D_2O

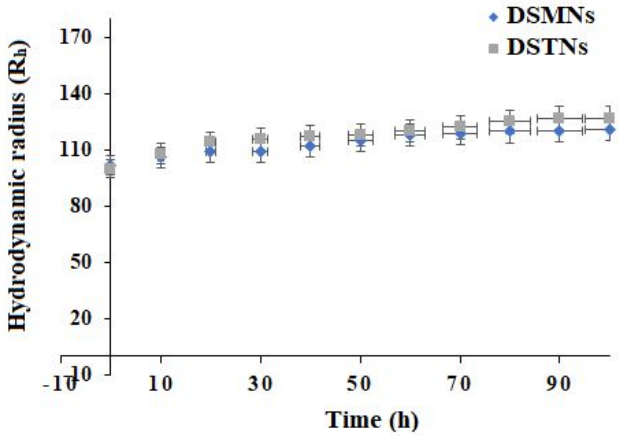

Figure S2. Hydrodynamic radius (R_h) of DSMNs and DSTNs at 289 K.

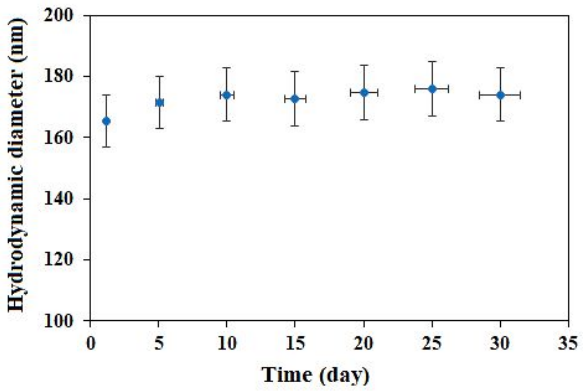

Figure S3. Change in hydrodynamic diameter for the PEI- FA-DSTNs in physiologic medium that measured by DLS.

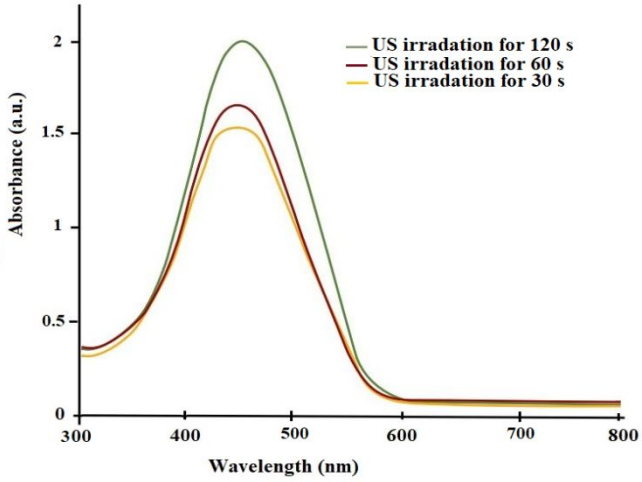

Figure S4. The stability of curcumin under differet amounts of reactive oxygen species in PBS.

MTT assay

To investigate the effect of US irradiation on cancer cells viability, the MTT assay was performed. The cytotoxic activity of US irradiation was studied against Hela and A549 cancer cell lines for 30, 60, and 120 s. HeLa and A549 cells free from US irradiation were used as control groups.

The obtained results from the MTT assay showed that, compared with the control groups, the proliferation of both cancer cells was inhibited when they subjected to ultrasonic irradiation (Figure S5). Moreover, the results indicated that, when the ultrasonic irradiation times were 30 and 60 s cell proliferation was inhibited, although without statistical significance. When the ultrasonic irradiation time was increased to 120 s, the inhibiting effect was improved compared with the other groups. As a result, in a certain range, the enhancing effect was gradually elevated with increasing irradiation time from 30 to 120 s. According to the previous research, the main reason for this phenomenon is hydrodynamic stress (sonomechanical process), inertial cavitation (pyrolytic process) and also temperature. changes induced by sonication.

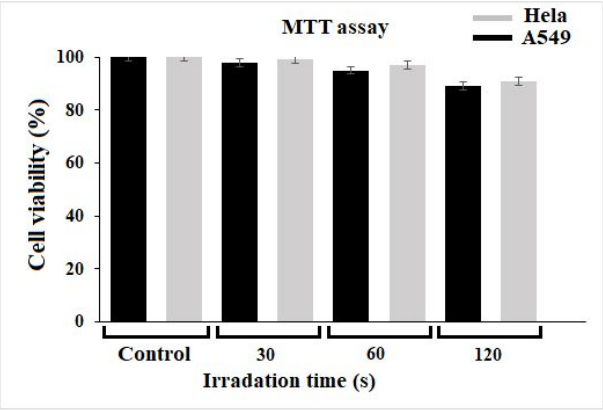

Figure S5. The cytotoxic effect of ultrasonic irradiation for 30, 60 and 120s against Hela, and A549 cancer cell lines.

The *in vitro* cytotoxic activity of the PEI-FA-DSTNs nanocarrier, at the concentration range of 1–200 μg/mL under different US irradiation times was studied against two human cancer cell lines, including Hela (FR-positive) and A549 (FR-negative) and the results are shown in Figure S6. According to the obtained results, all the treatments showed a dose-dependent cytotoxic activity in both cell lines. The cell inhibition activity for Hela cells, treated with PEI-FA-DSTNs under US irradiation for 30, 60, and 120 s, is increased to 21, 27, and 35%, respectively. According to the MTT results, in the presence of US irradiation, the cell viability is decreased with an increase in the US irradiation time. As we expected in the presence of US irradiation and TiO_2 nanoparticles as sonosensitizer agents, free radicals ($OH^{\bullet}$ and O_2^{-}) formation increased, and caused more cytotoxic activity.

In addition, the cytotoxic activity of A549 cells was investigated in the same experimental conditions and the results are shown in Figure S6. It can be seen that the cytotoxicity of nanocomposite becomes greater along with the increasing US irradiation time and also the concentrations from 1 to 200 μg/mL. According to the MTT results, the Hela cells with overexpressed folate receptors exhibit a higher cellular uptake of PEI-FA-DSTNs, which result in more cell apoptosis through a folate receptor-mediated endocytosis compared to the A549 cells.

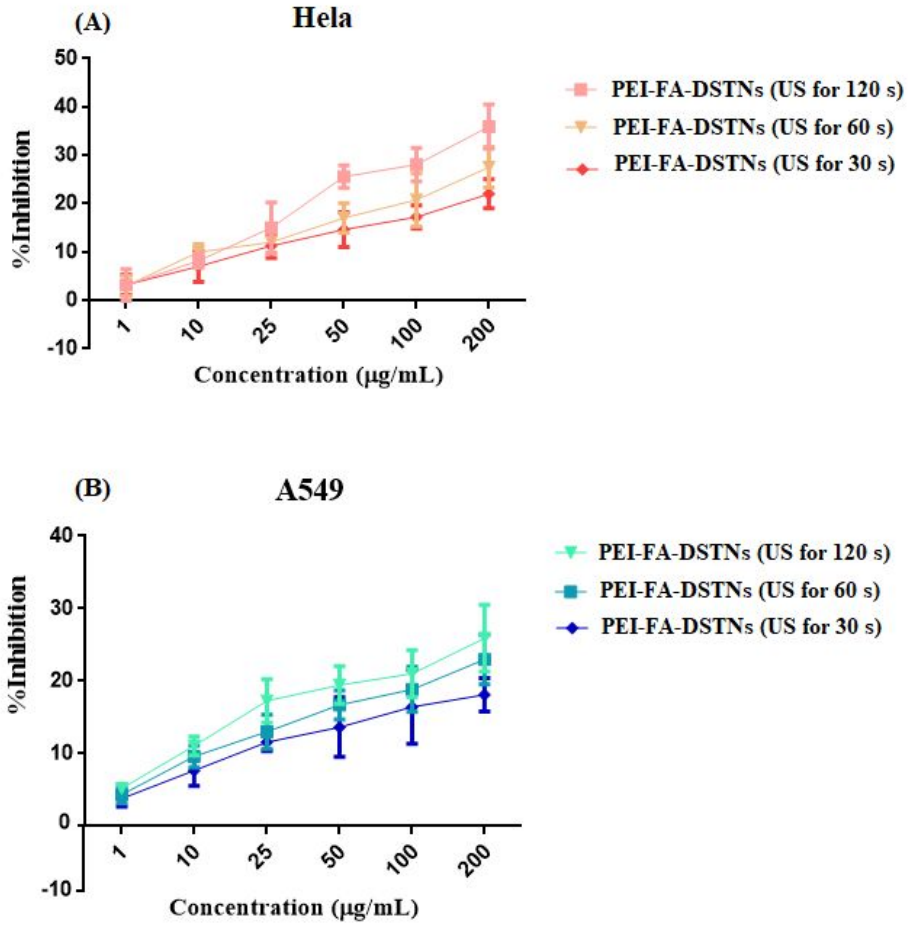

Figure S6. The cytotoxic effect of the PEI-FA-DSTNs nanocarrier under different US irradiation times against A) Hela, and B) A549 cancer cell lines in a concentration-dependent manner.