Supporting Information

Zwitterionic Carbon Dot-Encapsulated pH-Responsive Mesoporous Silica Nanoparticles for NIR Light-Triggered Photothermal Therapy through pH Controllable Release

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1. Experimental Section

1.1. Quantitative evaluation of Cellular Particle Uptake.

To quantified the cellular association of ICG-MSN(CD), The MDA-MB-231 and MDCK cells were cultured in a 48-well plate (1×10^5 cells/mL per well) and incubated in a humidified 5% CO_2 atmosphere for 24 h at 37°C. The cells were treated with ICG-MSN(CD) (0.5 mg/mL) dissolved in medium for 4 h. The media was removed and washed several times with PBS (pH 7.4). Triton X-100 (1%) was used to lyse the cells. The relative amount of ICG-MSN(CD), accumulated within the cells was determined by measuring the fluorescence intensity at an excitation wavelength of 365 nm and an emission wavelength of 465 nm, using the FilterMax F3 multi-mode microplate reader (Molecular Devices LLC.). The in vitro cellular uptake was calculated relative to ICG-MSN(CD) initial total concentration (100%, used as control).

Flow cytometry was conducted to analyse cellular uptake of nanoparticles. MDCK and MDA-MB-231 cells were grown in 6-well plates at a density of 1×10^6 cells/mL per well and incubated in humidified 5% CO_2 for 24 h at 37°C. After incubation, the medium was replaced with a solution of ICG-MSN(CD) dissolved in medium and incubated. After a designated time, the cells were collected and 1% FBS diluted with PBS (pH 7.4) was added. The mixture was analysed using the Attune NxT Acoustic Focusing Cytometer at an excitation wavelength at 405 nm and emission wavelength of 440/50 nm.

1.2. Flow Cytometry of Intracellular Localization.

MDCK and MDA-MB-231 cells were cultured in 6-well plates at a density of 1×10^6 cells/mL per well and incubated in humidified 5% CO_2 for 24 h at 37°C. After incubation, the medium was replaced with a solution of ICG-MSN(CD) dissolved in medium and incubated again for a designated time. After the cells were collected, 1% FBS diluted with PBS (pH 7.4) was added,

and then the mixture was analysed using the Attune NxT Acoustic Focusing Cytometer at an excitation wavelength at 405 nm and emission wavelength of 440/50 nm for nanoparticle and SYTO 61 at an excitation wavelength 638 nm and emission wavelengths of 670/14 nm.

Table 1. The N_2 adsorption–desorption parameters before and after ICG loading.

	Surface Area (m^2/g)	Pore Volume (cm^3/g)	Pore Size (nm)
MSN(CD)	546.1	0.35	2.6
ICG-MSN(CD)	279.2	0.16	2.3

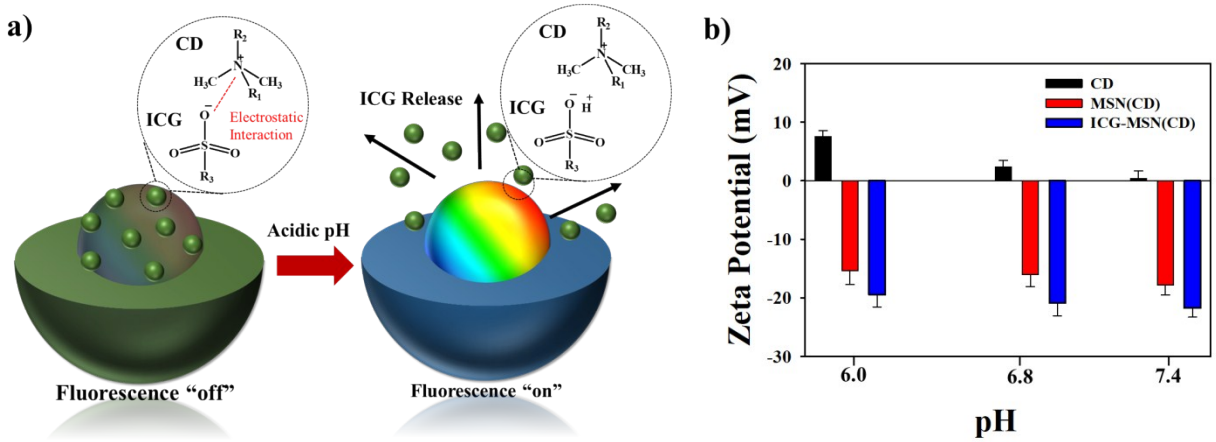

Figure S1. a) Schematic of fluorescence "off/on" system of ICG-MSN(CD) b) Zeta potential of CD, MSN(CD), and ICG-MSN(CD).

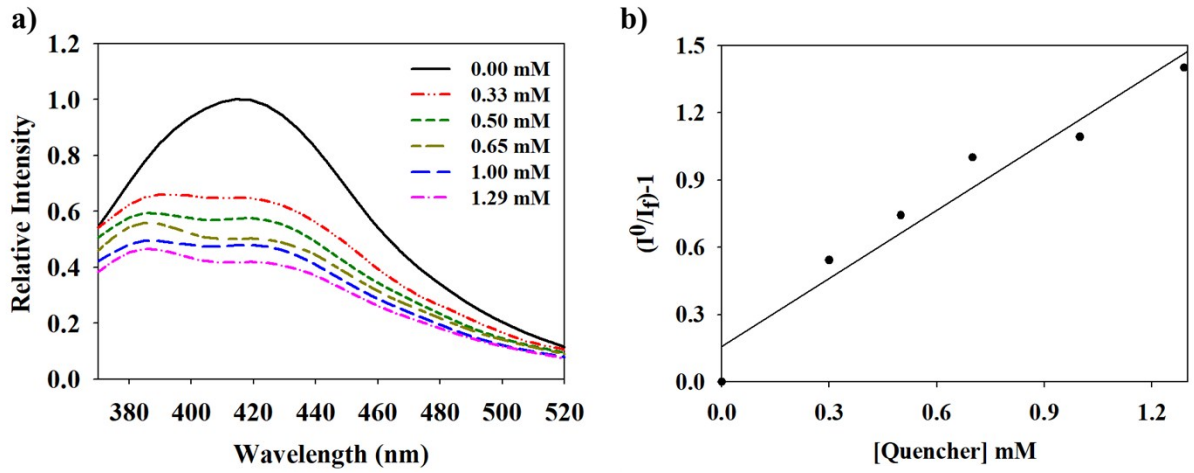

Figure S2. (a) Luminescence spectra of ICG-MSN(CD) with different concentrations of ICG at an excitation wavelength of 340 nm (b) Stern–Volmer plots for quenching of the luminescence spectra of ICG-MSN(CD) by ICG.

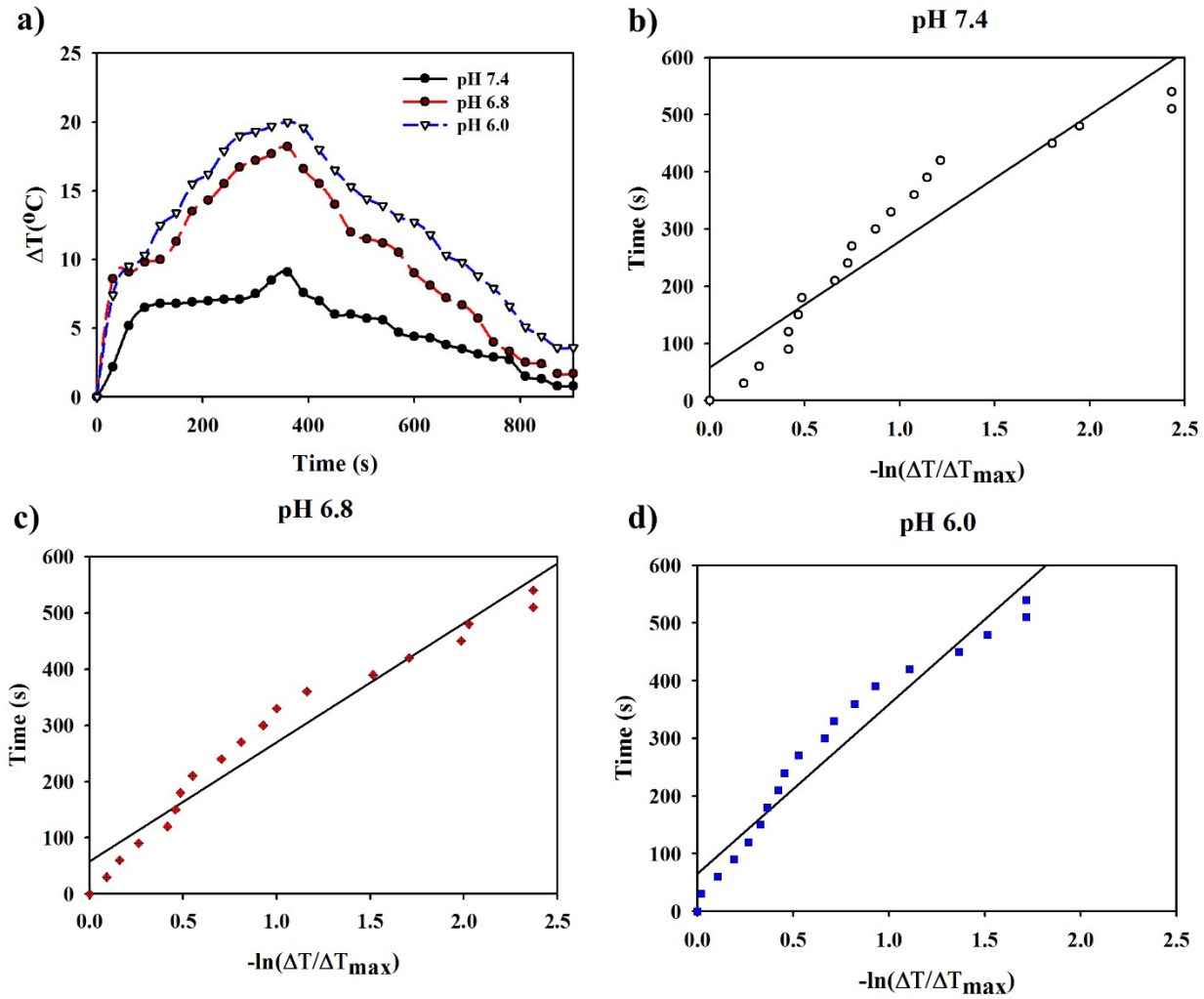

Figure S3. (a) Temperature profile of a ICG-MSN(CD) solution with different pH (1 mg/mL) when irradiated with an 808 nm laser (2 W/cm^2) during 6 min and after turning off of the laser during 9 min. Time constant for heat transfer is determined by applying the linear time from the cooling period (from 360 to 900 s) versus negative natural logarithm of the driving force temperature of (b) pH 7.4, (c) pH 6.8, and (d) pH 6.0.

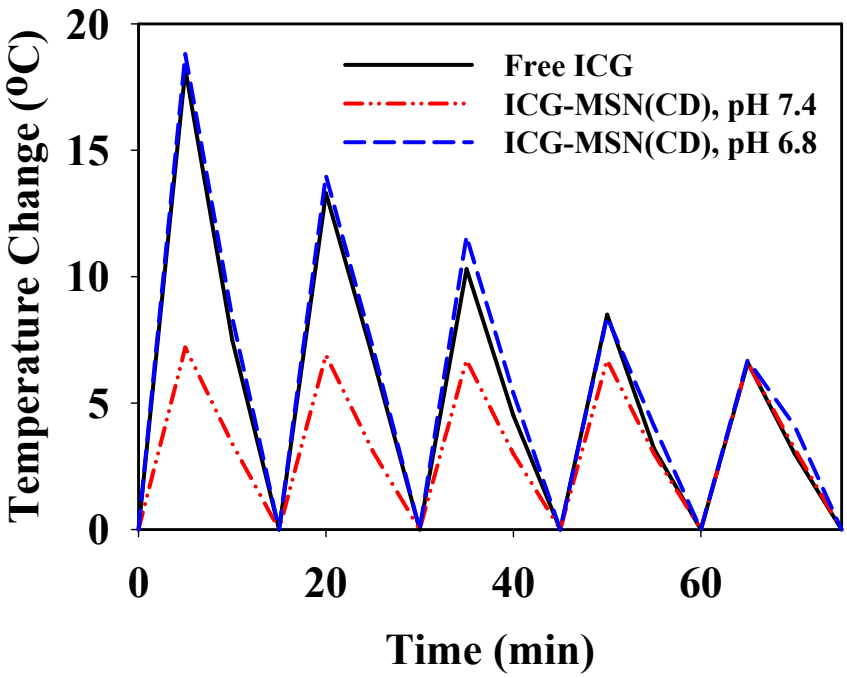

Figure S4. Temperature change of Free ICG and ICG-MSN(CD) at different pH over five NIR Irradiation on/off cycles (Laser on for 5 minutes and naturally cooling down for 10 minutes).

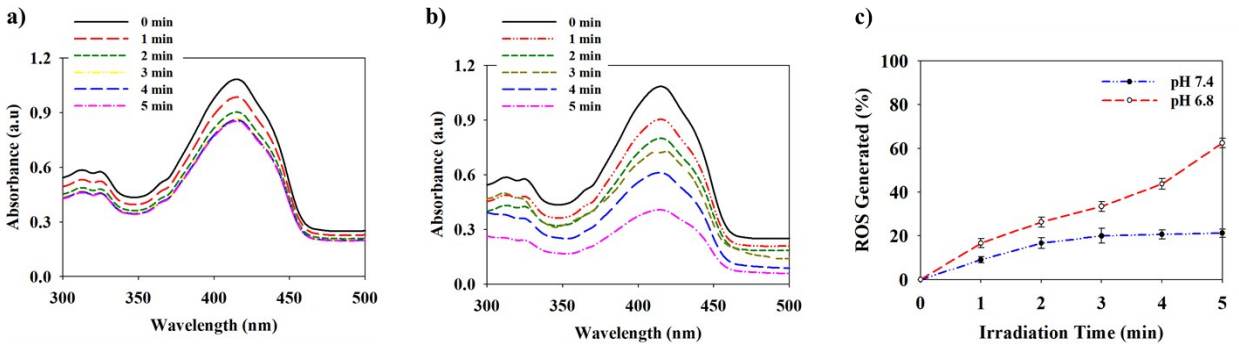

Figure S5. UV-Vis Spectra of DPBF and ICG-MSN(CD) treated at pH (a) 7.4 and (b) 6.8 irradiated with NIR laser 808 nm (power density of 2 W/cm^2) for various irradiation time. ROS generated-irradiation time correlation curve (c).

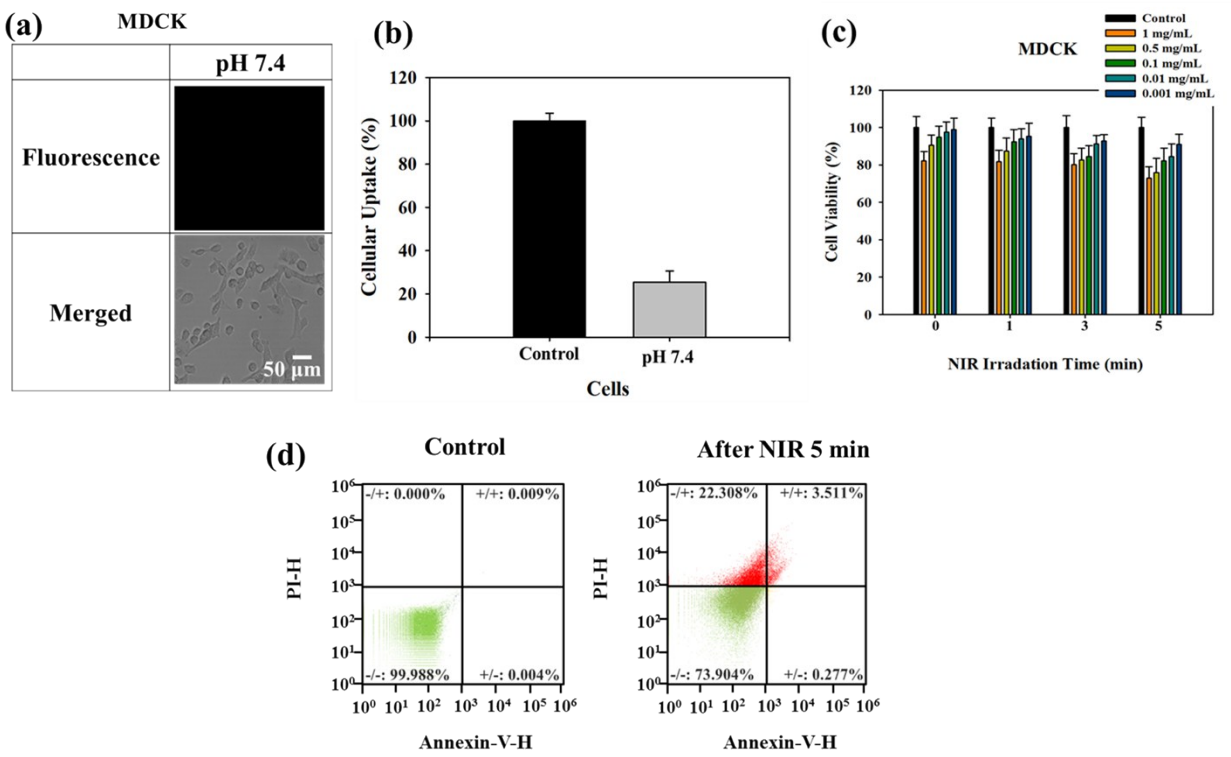

Figure S6. (a) Confocal microscopic fluorescence images of MDCK cells treated with ICG-MSN(CD) nanoparticles. (b) Cellular Uptake test of ICG-MSN(CD) by MDCK. (c) Cytotoxicity Test of ICG-MSN(CD) in MDCK cells (d) Flow cytometry of cell apoptosis of MDCK after ICG-MSN(CD) (concentration 1 mg/mL) uptake after 5 min irradiation using NIR laser 808 nm (power density of 2 W/cm^2).

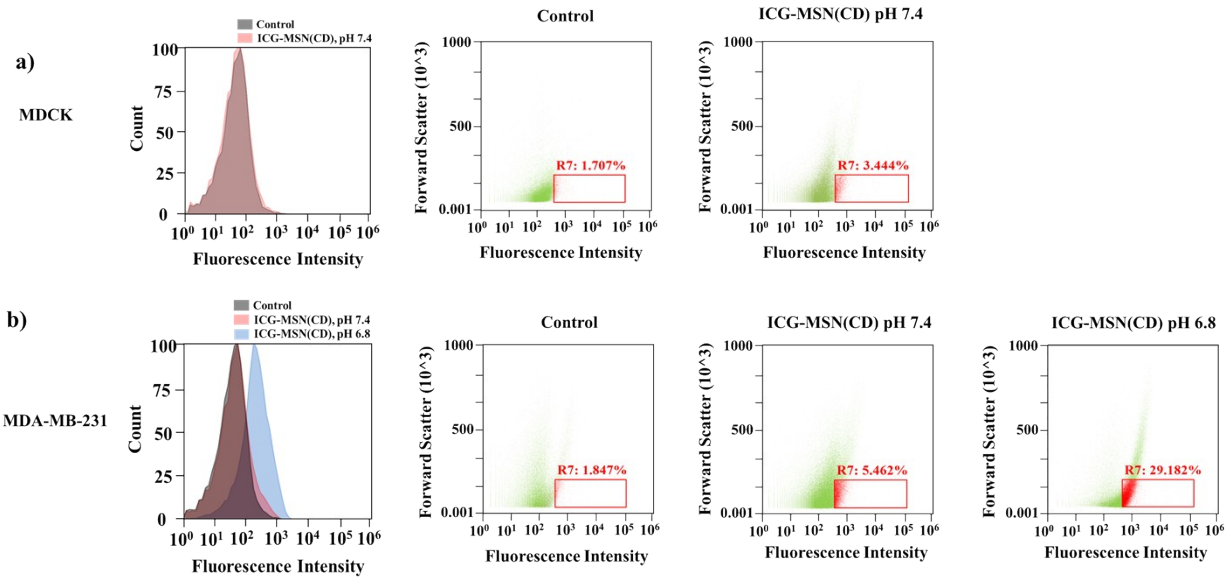

Figure S7. Cellular Uptake analysis using flow cytometry of MDCK (a) and MDA-MB-231 (b) after treated with ICG-MSN(CD) at different pH.

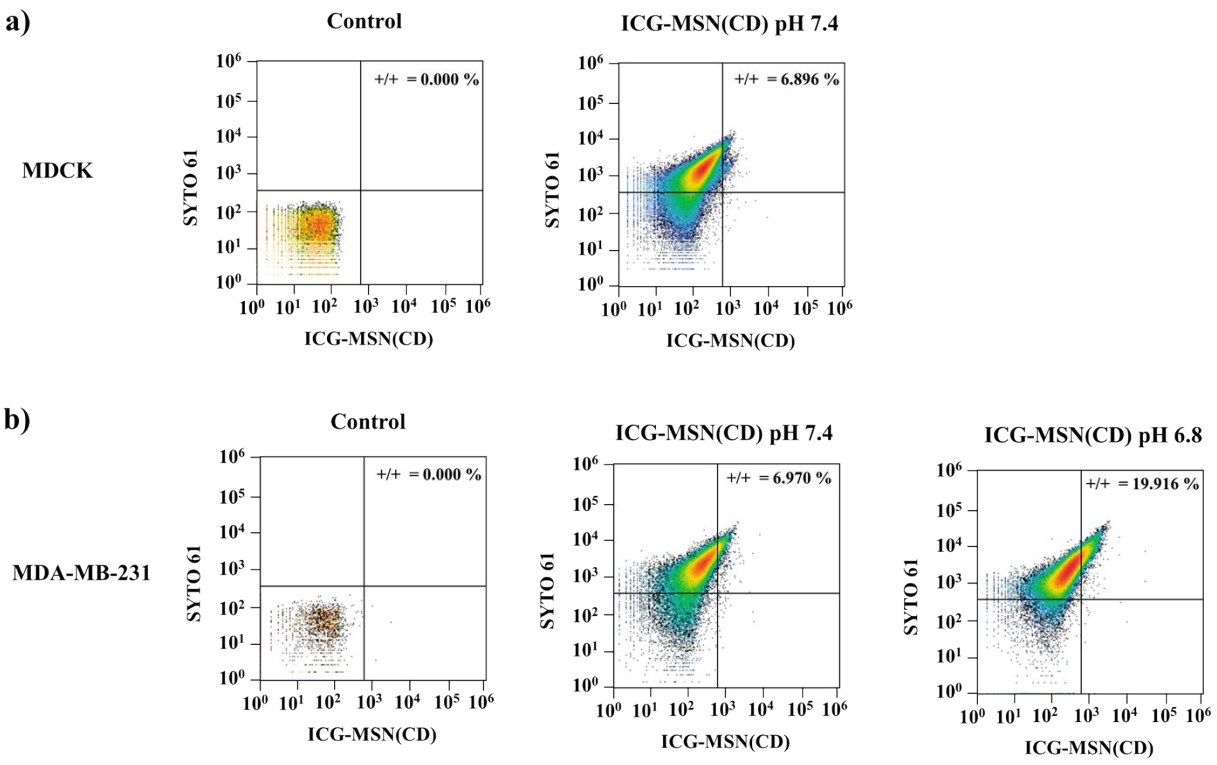

Figure S8. Cellular Internalization analysis using flow cytometry of MDCK and MDA-MB-231 after treated with ICG-MSN(CD) and stained with SYTO 61 (nuclear stain) at different pH.

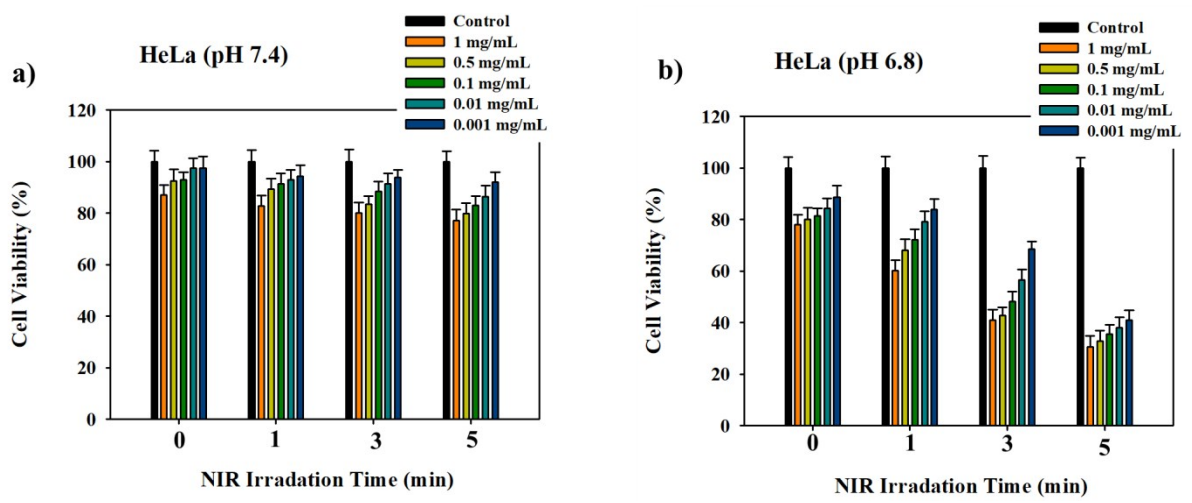

Figure S9. Cytotoxicity Test of ICG-MSN(CD) in HeLa cells at different pH (a) 7.4 and (b) 6.8 irradiated with NIR laser 808 nm (power density of 2 W/cm^2).

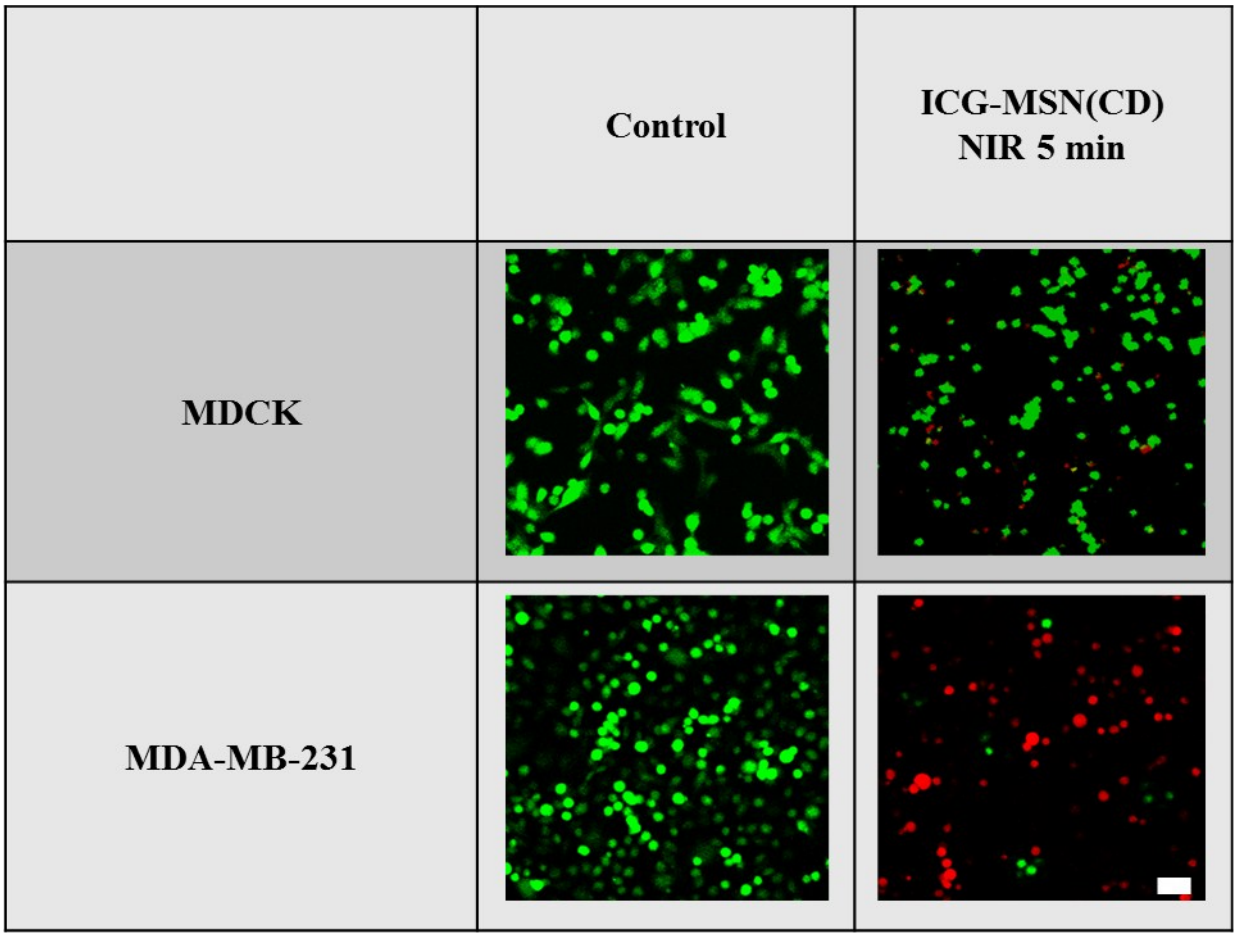

Figure S10. Live and Dead cell imaging spectroscopy of MDCK and MDA-MB after treatment with ICG-MSN(CD) and 5 min NIR Irradiation.