Supporting Information

NIR-activated quercetin-based nanogels embedded with CuS nanoclusters for the treatment of drug-resistant biofilms and accelerated chronic wound healing

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Experimental Section

Materials

Fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) were purchased from Gibco BRL (Grand Island, NY, USA). Trisodium citrate dihydrate ($Na_3C_6H_5O_7$. $2H_2O$), Sodium chloride (NaCl) and 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO ≥97%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous copper (II) chloride ($CuCl_2$) and hydrogen peroxide (aqueous solution, 35%) were procured from Showa Chemical Co. Ltd., (Tokyo, Japan). Horseradish peroxidase enzyme were purchased Tokyo Chemical Industry Co. Ltd., (Tokyo, Japan). Sodium hydroxide (NaOH) was purchased from J.T. Baker (Center Valley, PA, USA). L-Ascorbic acid was bought from Merck (Kenilworth, NJ, USA). Quercetin dehydrate (99%), sodium sulfide non-hydrate ($Na_2S{\cdot}9H_2O$), sodium phosphate dibasic heptahydrate (Na_2HPO_4 $\cdot 7H_2O$), sodium phosphate monobasic monohydrate ($NaH_2PO_4{\cdot}H_2O$), and poly(ethylene glycol) (Average M.W. 8k) were purchased from Acros Organics (Geel, Belgium). The ELISA Kits for mouse cytokine (IL-1β, IL-10, and TGF-β1) were ordered from R&D systems (Minneapolis, MN, USA). Bacterial strains [*P. aeruginosa* (ATCC 27853), *S. aureus* (BCRC10781), *E. coli* (BRBC 12438), *S. enteritidis* (BRBC 10744), and MRSA (methicillin-resistant *Staphylococcus aureus*, (ATCC 43300)] were acquired from the Institute of Food Science (Hsinchu, Taiwan). Milli-Q ultrapure water (18.2 MΩ cm) from Millipore (Billerica, MA, USA) was used in all experiments

Characterization of CNGs

Functional groups present in quercetin, Qu–CNGs, and CuS/Qu–CNGs were identified by Fourier transform infrared (FTIR) spectroscopy using Nicolet iS5 FTIR spectrometer (Thermo Scientific, Waltham, MA, USA). High-resolution transmission electron microscopy (HR-TEM) images of Qu–CNGs and CuS/Qu–CNGs were obtained using field emission transmission electron microscopy (FE-TEM) equipped with dual focused ion beam system (FIB) and energy dispersive X-ray spectrometer (EDS). Each of the CNGs was diluted ~1000-fold in ultrapure water, from which 1.0 μL was dropped onto a carbon-coated grid and vacuum dried for 72 h at ambient temperature before measurements. X-ray diffraction patterns for Qu–CNGs and CuS/Qu–CNGs was

recorded using X-ray source (SP12B1; energy range: 8-25 KeV) from a bending magnet and a CCD detector (BL12B2, SPring-8, and a 3-pin plate diamond anvil cell) from Almax Easy Lab (Almax Industries, Diksmuide, Belgium). The two-dimensional images were then merged using the FIT2D program with "Appendix" to fix the wavelength corresponding to Cu Kα (1.5418 Å). UV-Vis-NIR absorption spectra of the Qu–CNGs and CuS/Qu–CNGs in the range of 200-1100 nm were recorded with a double-beam Jasco UV/VIS/NIR spectrophotometer (V-570, Easton, MD, USA). The binding energies of the elements C, O, Cu, and S were obtained from X-ray photoelectron spectroscopy (XPS, VG ESCA210) from VG Scientific (West Sussex, UK). All the binding energies were corrected using C 1s (284.6 eV) as an internal standard. 10 µL of Qu–CNGs and CuS/Qu–CNGs onto Si substrate were dried separately at ambient temperature for 12 h prior to XPS analysis.

Determination of Total Phenolic Content

Confirmatory test for the presence of phenol or polyphenol in Qu–CNGs and CuS/Qu–CNGs was conducted using Folin-Ciocalteu (F-C) reagent. In a typical assay 5.0 µg mL^{-1} (in terms of Qu) of monomeric Qu (mQu; dissolved in DMSO), Qu–CNGs, CuS/Qu–CNGs, and ascorbic acid (positive control) were separately incubated with F-C reagent (0.0125 N) in 10 mM sodium phosphate buffer (pH 7.4) for 5 min at room temperature, prior to the addition of Na_2CO_3 (0.1N). Aliquots were then incubated in the dark for 1 h under orbital shaking (150 rpm) and 200 µL from each mixture was subjected to the absorption measurement at 765 nm (Abs_{765}) using a monochromatic microplate spectrophotometer (Synergy 4, Biotek Instruments, Winooski, VT, USA).

Enzyme-Like Activities of CuS/Qu–CNGs

To investigate the oxidase-like activity, a freshly prepared Amplex Red (AR; 10 µM) solution was incubated separately with 5.0 µg mL^{-1} (in terms of Qu) of mQu, Qu–CNGs and CuS/Qu–CNGs dispersed in 10 mM sodium phosphate buffer (pH 7.4) at ambient temperature for 1 h under dark. Fluorescence intensity of each solution at 590 nm was measured with a fixed excitation wavelength at 540 nm.

The peroxidase-like activities of mQu, Qu–CNGs and CuS/Qu–CNGs at a concentration of 5.0 µg mL^{-1} (in terms of Qu) dispersed in 10 mM of sodium phosphate

buffer (pH 7.4) were evaluated using AR (10 μM) as a substrate, in the presence of H_2O_2 (1.0 mM). Each of the solution was incubated for 1 h at ambient temperature and AR reacted with HRP (0.1 unit mL^{-1}) served as a positive control. 200 μL from each mixture was transferred to 96-well flat bottom plate to record the fluorescence intensity at 590 nm at an excitation wavelength of 540 nm.

Bacterial Viability Assays

E. coli suspensions (2.0×10^8 CFU mL^{-1}) were treated with 0.2 μg mL^{-1} (in terms of Qu) of Qu–CNGs or CuS/Qu–CNGs in the presence and absence of H_2O_2 (10 μM) in 10 mM sodium phosphate buffer (pH 7.4) for 1 h. Afterwards, each of the solution was incubated without or with NIR-II laser (1064 nm, 0.94 W Cm^{-2}) at ambient temperature for 10 min. The bacterial suspensions were then centrifuged (RCF 3000 *g*, 5 min, 27 °C) and washed thrice with 10 mM sodium phosphate buffer (pH 7.4) before fluorescence observation. A LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, Eugene, OR, USA) was used to evaluate the live/dead bacteria ratio in each of the mixture. Briefly, 2.0 μL of SYTO 9: PI (1:1) mixture was incubated with 0.5 mL of untreated and treated bacterial samples for 20 min at ambient temperature under dark. Unbound dye was removed by centrifugation (RCF 3000 *g*, 5 min, 27 °C) and washed thrice with sodium phosphate buffer (10 mM, pH 7.4, 1.0 mL). The purified bacterial samples (1.0 μL) were fixed with Fluoromount aqueous mounting medium from Sigma-Aldrich (St. Louis, MO, USA) on a glass slide for fluorescence observation. The live/dead bacteria were stained with green/red fluorescence, respectively, and the images were captured using an excitation filter at 460–490 nm under an Olympus 1X71 microscope (Tokyo, Japan).

Reactive Oxygen Species (ROS) Assays

E. coli suspensions (2.0×10^8 CFU mL^{-1}) were reacted with 0.2 μg mL^{-1} (in terms of Qu) of Qu–CNGs or CuS/Qu–CNGs in the presence and absence of H_2O_2 (10 μM) in 10 mM sodium phosphate buffer (pH 7.4) for 1 h. Then, all the bacterial mixtures were treated without or with NIR-II laser (1064 nm, 0.94 W cm^{-2}) at ambient temperature for 10 min. The solutions were then centrifuged (RCF 3000 *g*, 5 min, 27 °C) and washed thrice using 10 mM sodium phosphate buffer (pH 7.4, 1.0 mL). Untreated and H_2O_2 (1.0 mM) -treated bacteria served as the negative and positive controls, respectively. To each

of the bacterial sample 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, 100 μM) was separately added and incubated at room temperature for 2 h under dark condition. Eventually, 200 μL from each aliquot was transferred into a 96-well flat-bottom microplate to estimate the fluorescence intensities at 530 nm, with a fixed excitation at 490 nm.

Bacterium Membrane Potential

E. coli suspensions (2.0×10^8 CFU mL^{-1}) were incubated with 0.2 μg mL^{-1} (in terms of Qu) of Qu–CNGs or CuS/Qu–CNGs in the presence and absence of H_2O_2 (10 μM) dispersed in 10 mM sodium phosphate buffer (pH 7.4, 1.0 mL) for 1 h. Each of the aliquot was treated without or with NIR-II laser (1064 nm, 0.94 W cm^{-2}) at ambient temperature for 10 min. Bacterial solution treated with PBS and B-PER lysis buffer were used as positive and negative controls, respectively. Each of the aliquot (1.0 mL) was then centrifuged (3000 *g*, 5 min, 27 °C) to remove the bacteria (pellet). Obtained supernatants were then treated separately with *o*-nitrophenyl-*β*-D-galactopyranoside (ONPG, 200 μM) at ambient temperature for 2 h with orbital shaking (150 rpm). Lastly, 200 μL of each bacterial suspension was poured in a 96-well flat-bottom microplate to measure absorption at 420 nm (Abs_{420}).

TEM Images of Bacteria

E. coli (2.0×10^8 CFU mL^{-1}) suspensions were incubated with CuS/Qu–CNGs (0.2 μg mL^{-1}, in terms of Qu) in the presence of H_2O_2 (10 μM) in sodium phosphate buffer (10 mM, pH 7.4) for 2 h at ambient temperature. Resulting solutions treated without or with NIR-II laser (1064 nm, 0.94 W Cm^{-2}, 10 min) at ambient temperature for 10 min were subjected to centrifugation (3000 *g*, 5 min, 27 °C) to remove the unreacted CNGs. Resuspended bacterial cells (2.0 μL) were immobilized onto a carbon-coated copper TEM grid for 15 min at ambient temperature, after which the excess solution was sucked out, and the grid was dried under vacuum for 2 h before measurement.

In Vitro Cytotoxicitic Assays

The cytotoxicity of Qu–CNGs and CuS/Qu–CNGs was evaluated using the Alamar blue assay (Thermo Fisher Scientific Inc.). In a typical assay, HaCaT cells were seeded in 96-well plates (1×10^4 cells per well) and grown in a Dulbecco's modified Eagle's medium

(DMEM) in a 5% CO_2 at 37 °C for 12 h. Various concentrations (0-500 μg mL^{-1}) of Qu–CNGs and CuS/Qu–CNGs were incubated with the cells for 1 h at 37 °C with/without under irradiation with a NIR-II laser (1064 nm, 0.94 W Cm^{-2}, 10 min) then replaced with the fresh DMEM (10% FBS) to further grow for 24 h. Afterwards, the culture media was removed, and 100 μL of Alamar blue solution (1x) premixed with DMEM (10% FBS) was added to each well and incubated at 37 °C for 2 h. The fluorescence intensity from each well was recorded at 600 nm upon excitation at 550 nm using a microplate reader. The relative cell viability (%) was estimated using the following equation:

Cell viability (%) = $(F_{CNGs}/F_{Blank}) \times 100\%$, where F_{CNGs} and F_{Blank} are the fluorescence intensities of the cells in the presence and absence of Qu–CNGs or CuS/Qu–CNGs.

Hemolysis Assays

Blood sample was drawn from the vein of a healthy 30-year-old female and collected in a sterile vial containing $Na_3C_6H_5O_7$. Red blood cells (RBCs) collection procedures were in accordance with institutional guidelines and relevant laws. The blood sample was immediately centrifuged (3000 *g*, 20 min, 4 °C) to remove the serum, and washed thrice with a PBS solution (pH 7.4). Various concentrations (0-500 μg mL^{-1}) of Qu–CNGs or CuS/Qu–CNGs were spiked into the vial containing RBCs (~ 4 vol.% of blood cells) and incubated for 10 min with/without under irradiation using a NIR-II laser (1064 nm, 0.94 W Cm^{-2}) at ambient temperature. RBCs treated with PBS solution (pH 7.4, 0% hemolysis, $Abs_{576\ Blank}$) and ultrapure water (100% hemolysis, $Abs_{576\ DI\ water}$) were used as negative and positive controls, respectively. Post treatment, each of the aliquot was centrifuged (3000 *g*, 10 min, 4 °C). Then, 200 μL of supernatant was carefully collected to measure the hemoglobin absorption at 576 nm (Abs_{576}). The percentage of hemolysis was determined using the following formula:

Hemolysis (%) = $[(Abs_{576\ CNGs} - Abs_{576\ Blank})/(Abs_{576\ DI\ water} - Abs_{576\ Blank})]$

In Vivo Biocompatibility

To examine *in vivo* cytotoxicity of CuS/Qu–CNGs, body weights of mice were monitored over 18 days (0, 3, 6, 9, 12, 15, and 18). Blood samples were also withdrawn from the vein of untreated and CuS/Qu–CNGs treated groups (w NIR-II and w/o NIR-II) after postoperative day 18 to estimate the relative levels of vital biochemical indicators ALT, AST, T-BIL, GGT, and BUN

using HITACHI 7180 medium-sized biochemistry automatic analyzer. Mice were sacrificed on postoperative day 18, and essential organs such as kidney, heart, liver, spleen, and lung were harvested for histological evaluation using H&E tissue staining. The tissue section incorporated in optimal cutting temperature compound (Sakura Finetek, Torrance, CA, USA) were cryosectioned at 6 μm thickness onto Superfrost Plus Slides (Thermo Scientific, New Hamisphire, USA) (n = 3). Then, the H&E stained organs were fixed with multimedium onto glass stide to capture images using Olympus 1X71 microscope (Tokyo, Japan).

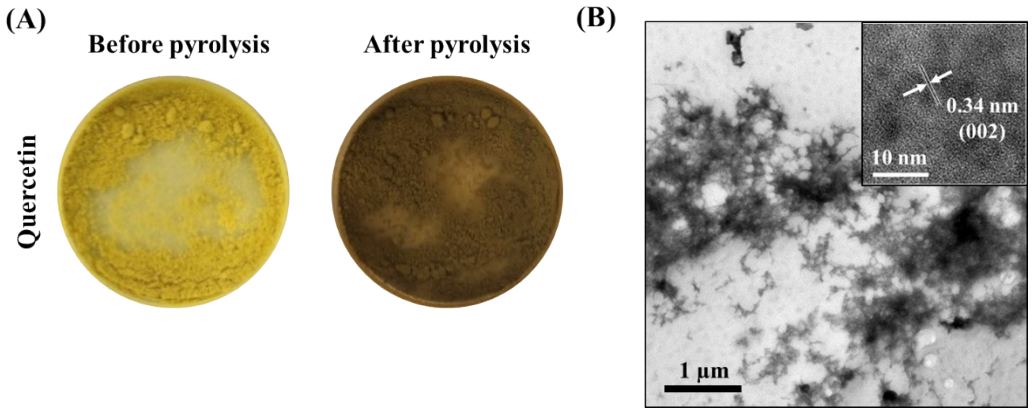

Fig. S1. (A) Photographs of the solid residue of quercetin before and after pyrolysis at 270 ºC for 2 h. (B) TEM image of Qu–CNGs. The insets show the corresponding HR-TEM image.

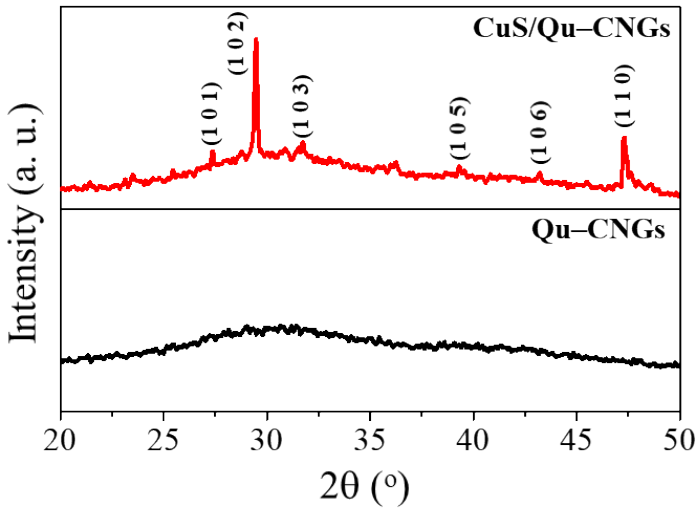

Fig. S2. X-ray diffraction patterns of the CuS/Qu–CNGs and Qu–CNGs. The XRD peaks are assigned to corresponding standards of CuS (JCPDS # 00-003-0724; Red).

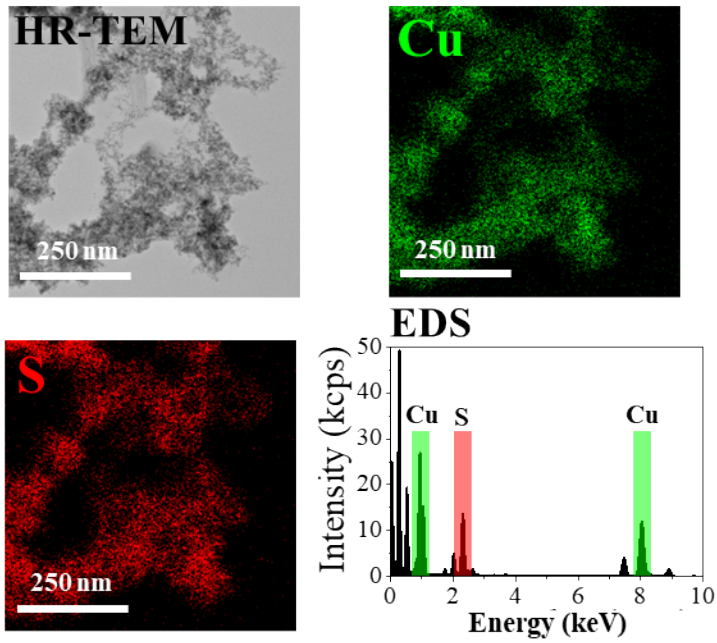

Fig. S3. TEM coupled elemental mapping images and EDS spectrum of CuS/Qu–CNGs.

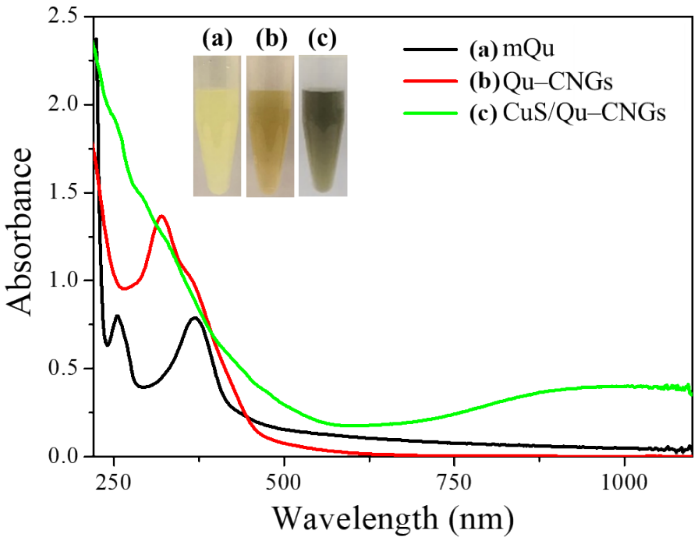

Fig. S4. UV-Vis-NIR absorption spectra of mQu, Qu–CNGs, and CuS/Qu–CNGs, diluted 10-fold in 10 mM sodium phosphate buffer (pH 7.4). Insets show the photographs of the corresponding solutions.

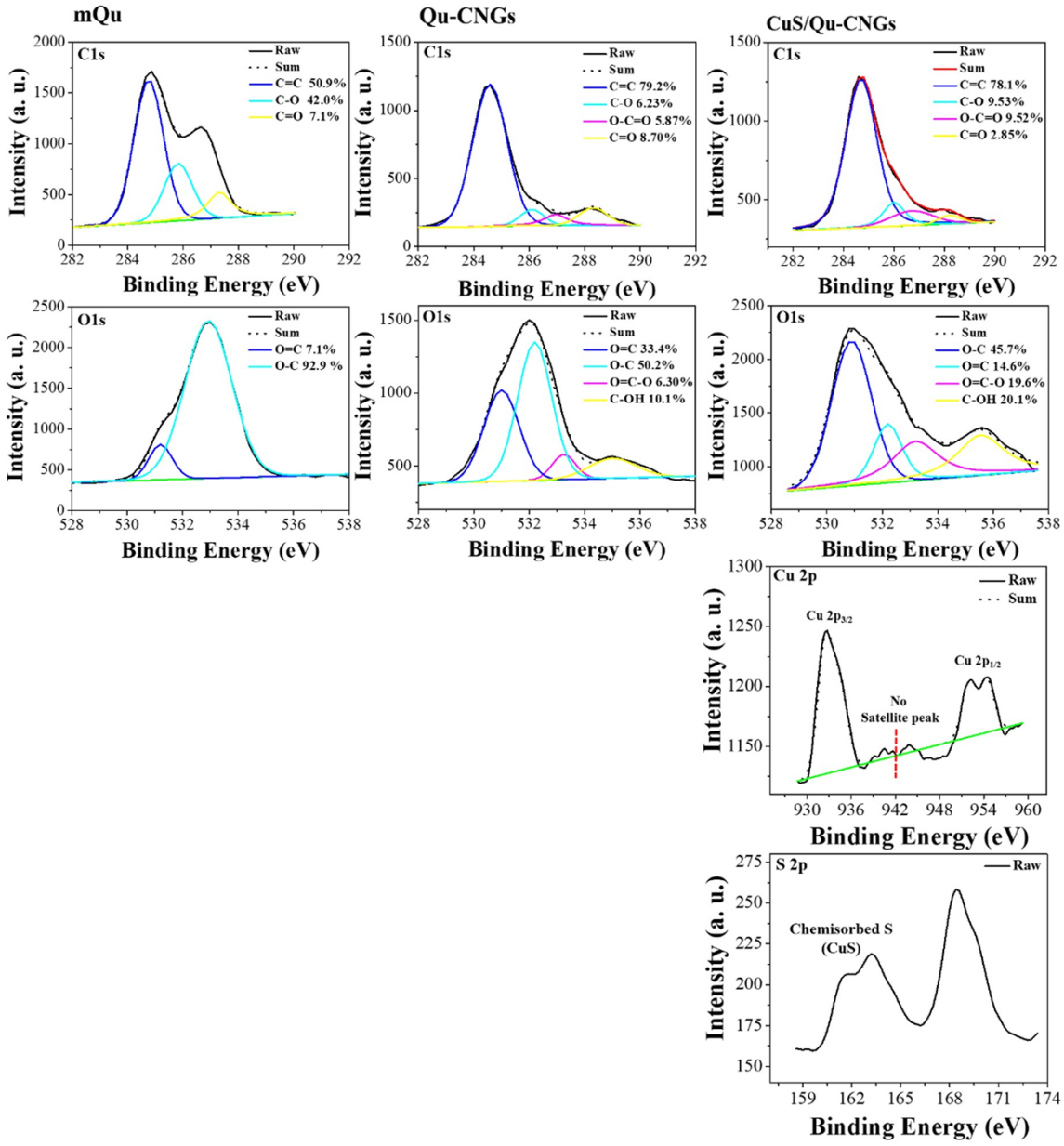

Fig. S5. Raw and deconvoluted XPS spectra of mQu, Qu–CNGs, and CuS/Qu–CNGs. The binding energies were corrected using C 1s (284.6 eV) as a standard.

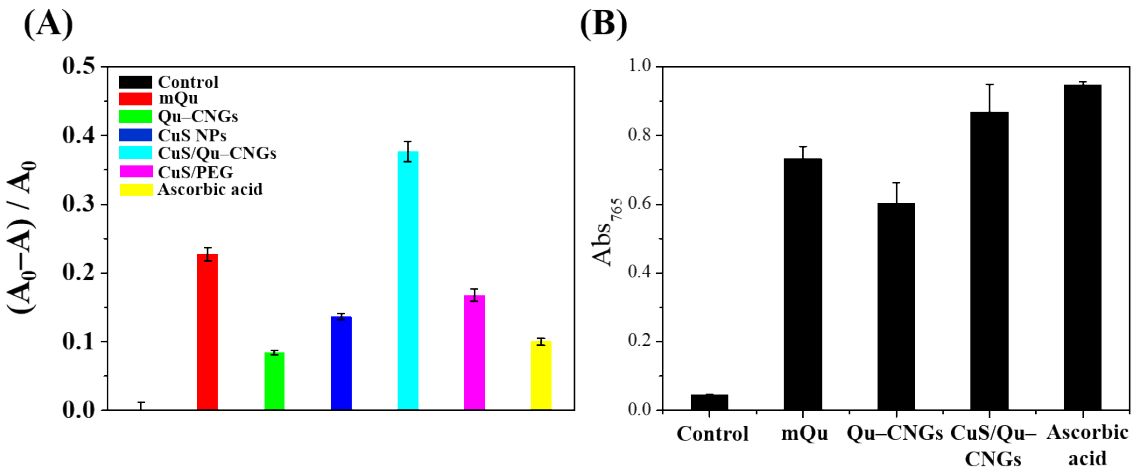

Fig. S6. (A) Relative absorbance [$(A_0 - A)/A_0$; at 517 nm] of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH; 200 µM) solutions before (control; A_0) and after (A) incubation with mQu (5.0 µg mL^{-1}), Qu–CNGs (5.0 µg mL^{-1}), CuS/Qu–CNGs (5.0 µg mL^{-1}; in terms of Qu–CNGs) or ascorbic acid (5.0 µg mL^{-1}) for 1 h. (B) The absorbance of Folin-Ciocâlteu reagent (0.0125 N) at 765 nm (Abs_{765}) solutions untreated (control) and incubated with, mQu (5.0 µg mL^{-1}), Qu–CNGs (5.0 µg mL^{-1}), or CuS/Qu–CNGs (5.0 µg mL^{-1}; in terms of Qu–CNGs) or ascorbic acid (5.0 µg mL^{-1}) for 1 h, in the presence of Na_2CO_3 (0.1 M). Error bars represent the standard deviations of three repeated measurements.

The Folin-Ciocalteu assay is based on the transfer of electrons from phenolic compounds in basic solution to phosphomolybdic/phosphotungstic acid to form blue complexes [$(PMoW_{11}O_{40})^{4-}$], which are quantitated by monitoring the absorbance at 765 nm (Abs_{765}). However, the Folin-Ciocalteu assay is sensitive not only to phenolic compounds but also to many reducing agents. Thus, this assay could be employed to measure the total reducing capacity of a sample, not only its phenolic compounds.

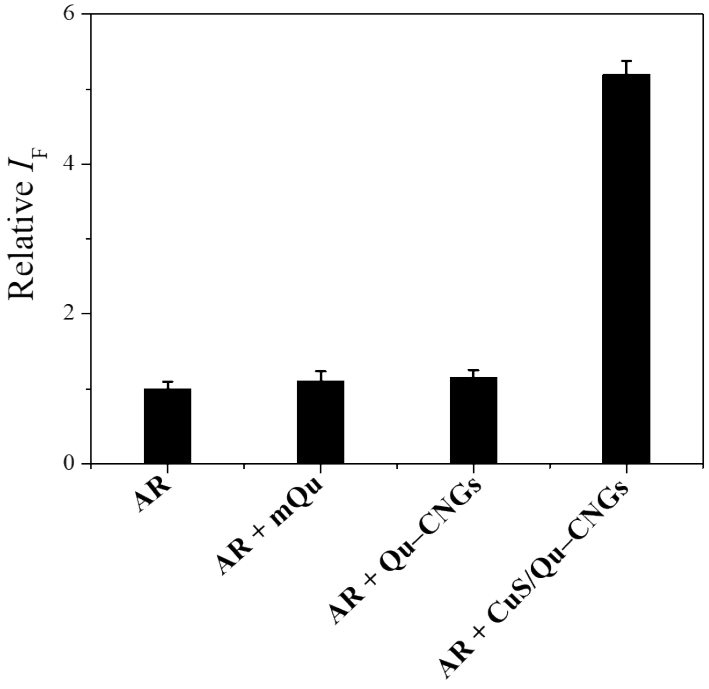

Fig. S7. Relative fluorescence intensities of AR (10.0 μM) in 10 mM sodium phosphate buffer (pH 7.4) before and after incubation with of mQu (5.0 μg mL^{-1}), Qu–CNGs (5.0 μg mL^{-1}) or CuS/Qu–CNGs (5.0 μg mL^{-1}; in terms of Qu–CNGs) for 1 h. The fluorescence intensities (I_F) were recorded at excitation/emission wavelengths of 530/590 nm. Error bars represent the standard deviations of three repeated measurements.

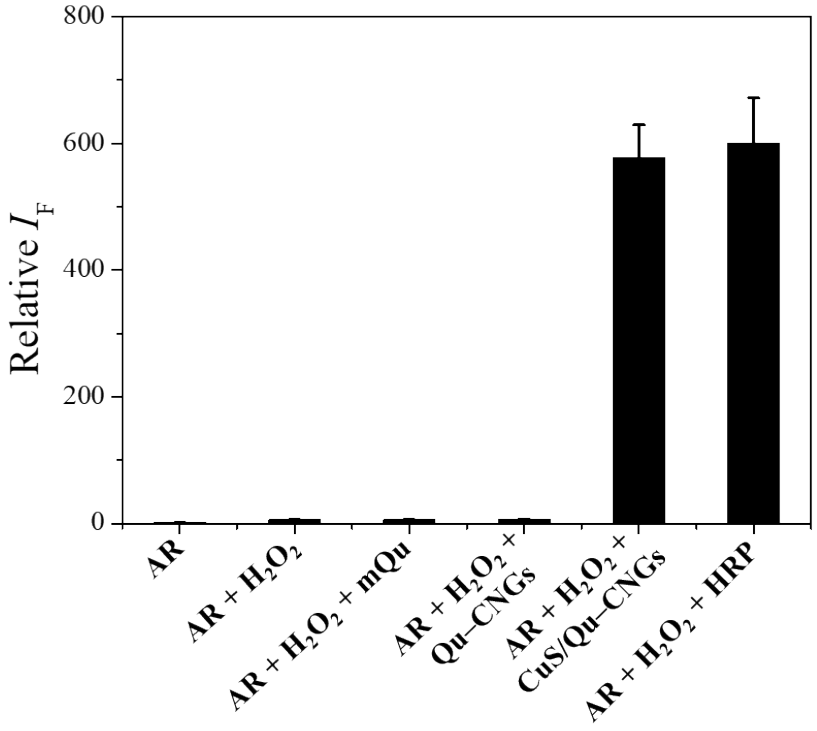

Fig. S8. Relative fluorescence intensities of AR (10.0 μM) in 10 mM sodium phosphate buffer (pH 7.4) before and after incubation with mQu (5.0 μg mL^{-1}), Qu–CNGs (5.0 μg mL^{-1}), CuS/Qu–CNGs (5.0 μg mL^{-1}; in terms of Qu–CNGs) or HRP (0.1 unit mL^{-1}) in the presence of H_2O_2 (1.0 mM). The fluorescence intensities (I_F) were recorded at excitation/emission wavelengths of 530/590 nm. Error bars represent the standard deviations of three repeated measurements.

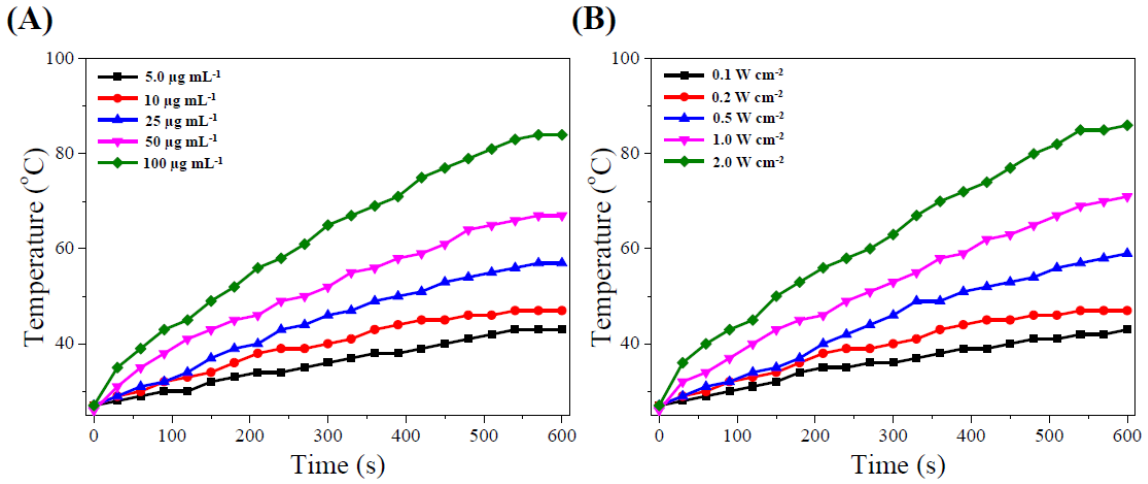

Fig. S9. (A) Concentration- and (B) power density-dependent temperature profiles of CuS/Qu–CNGs in sodium phosphate buffer (10 mM, pH 7.4) solution. Power density of the 1064 nm NIR-II laser in (A) is 0.94 W cm^{-2} and the concentration in (B) is 50 μg mL^{-1} (in terms of Qu-CNGs).

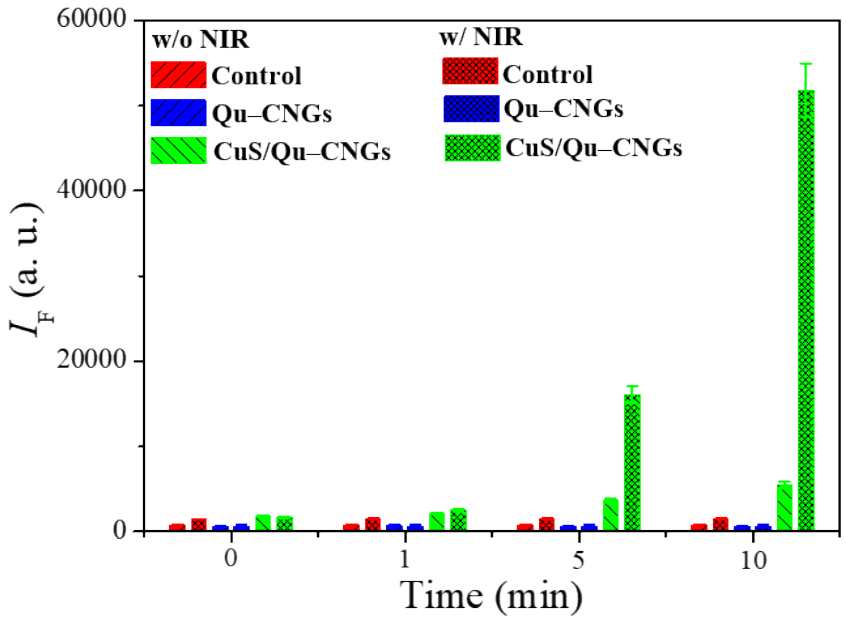

Fig. S10. Time-course fluorescence intensities (I_F) of AR (10 μM) in the absence and presence of Qu–CNGs (50 μg mL^{-1}) or CuS/Qu–CNGs (50 μg mL^{-1}) in 10 mM sodium phosphate buffer (pH 7.4) without (w/o) or with (w/) NIR-II irradiation (1064 nm, 0.94 W cm^{-2}) at an excitation/emission wavelength of 530/590 nm. Error bars represent the standard deviations of three repeated measurements.

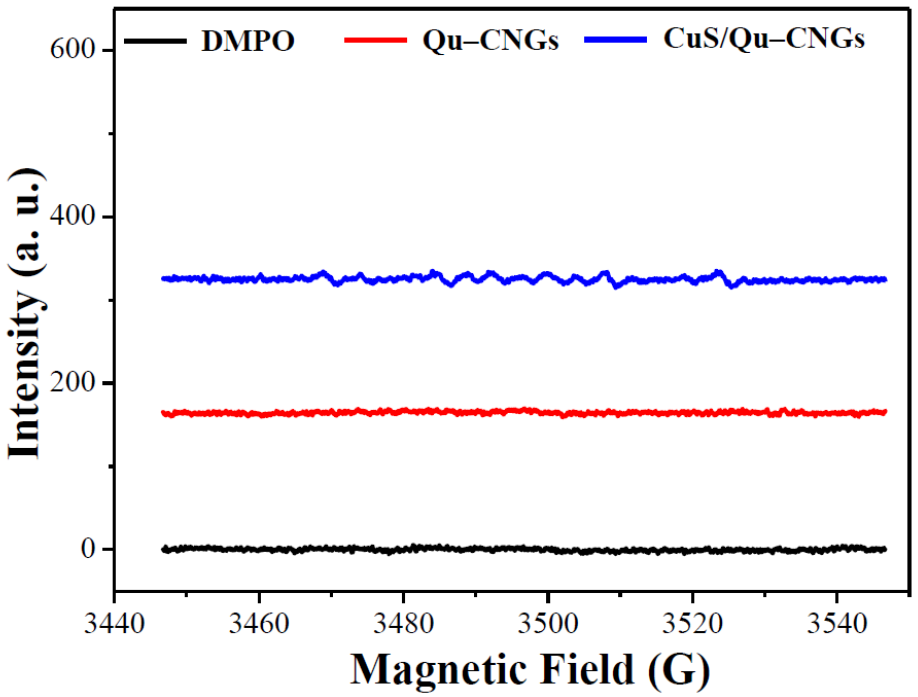

Fig. S11. ESR spectra of DMPO (10 mM) in sodium phosphate buffer (10 mM, pH 7.4) in the absence (as a control) and presence of 50 μg mL^{-1} of Qu–CNGs or CuS/Qu–CNGs without NIR-II laser irradiation (1064 nm, 0.94 W cm^{-2}, 10 min).

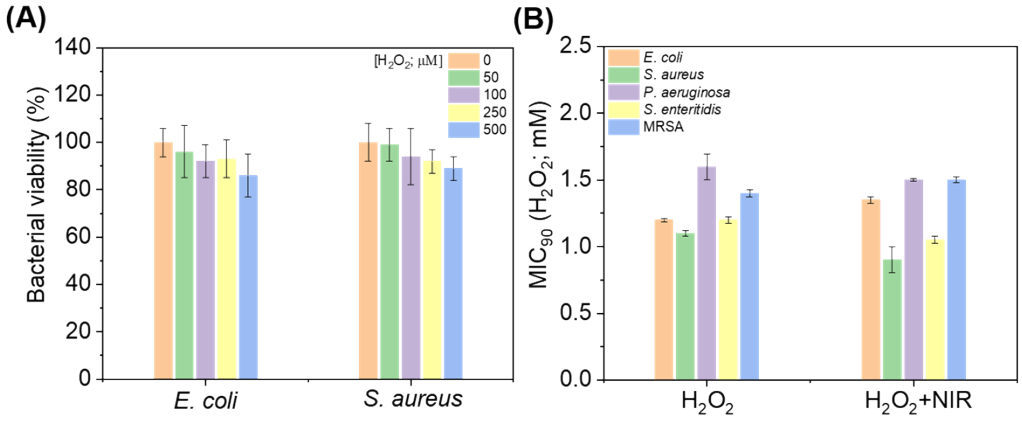

Fig. S12. (A) Relative bacterial viability of *E. coli* and *S. aureus* after incubation with various concentrations of H_2O_2 (0–500 μM). (B) MIC_{90} values of H_2O_2 without or with NIR-II laser (1064 nm, 0.94 W cm^{-2}) against five bacterial strains. Error bars represent the standard deviations of three repeated measurements.

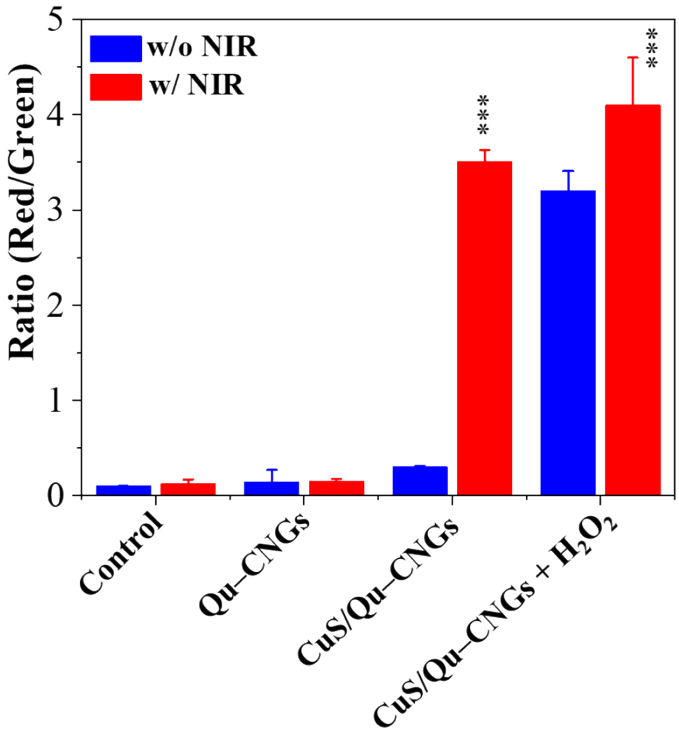

Fig. S13. Dead (red)/live (green) ratios from SYTO9/PI staining of *E. coli* (2.0×10^8 CFU mL^{-1}) in sodium phosphate buffer (10 mM, pH 7.4) untreated and treated with 0.2 μg mL^{-1} (in terms of Qu) of Qu–CNGs or CuS/Qu–CNGs in the absence and presence of H_2O_2 (10 μM) for 1 h without (w/o) or with (w/) NIR-II laser (1064 nm, 0.94 W cm^{-2}, 10 min) treatment. Error bars represent the standard deviations of three repeated measurements. Asterisks indicate statistically significant differences of the Qu–CNGs, CuS/Qu–CNGs, and CuS/Qu–CNGs+H_2O_2 groups compared to those of untreated ones, in the presence of NIR, respectively (***$p < 0.001$).

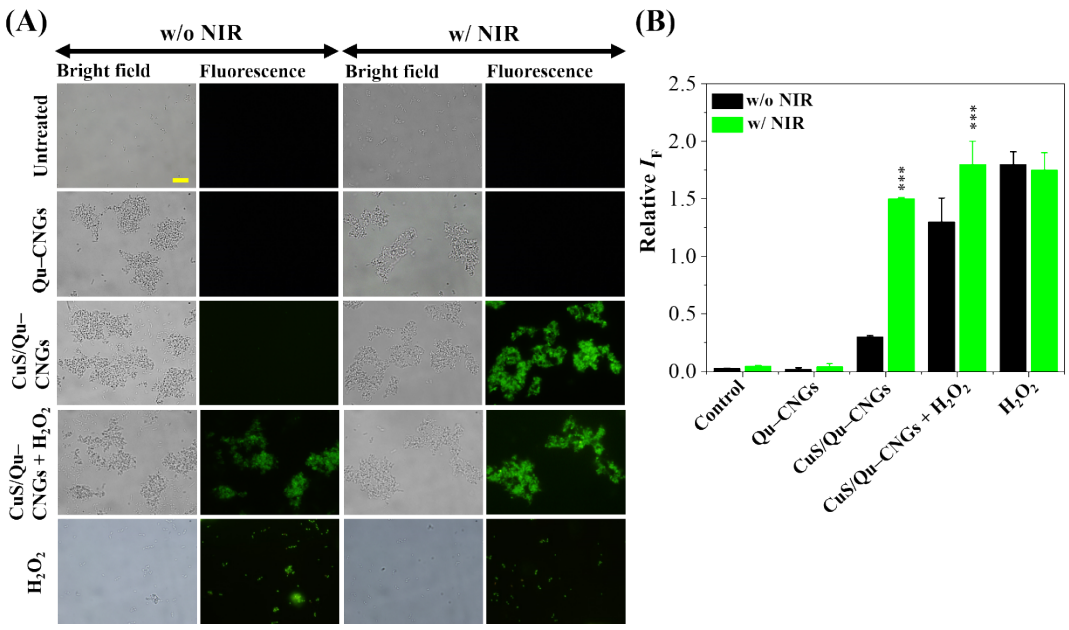

Fig. S14. (A) Bright field and fluorescence images and (B) relative fluorescence intensities of DCFH-DA stained *E. coli* (2.0×10^8 CFU mL^{-1}) untreated and treated with 0.2 μg mL^{-1} of Qu–CNGs or CuS/Qu–CNGs in the absence or presence of H_2O_2 (10 μM) in 10 mM sodium phosphate buffer (pH 7.4) for 1 h without (w/o) or with (w/) NIR-II irradiation (1064 nm, 0.94 W cm^{-2}, 10 min). The optical microscopy images were captured after the DCFH-DA staining. Untreated and H_2O_2 (1.0 mM) treated *E. coli* served as negative and positive controls, respectively. The scale bar in (A) is 10 μm. The error bars in (B) represent the standard deviation of four repeated measurements. Asterisks indicate statistically significant differences of the Qu–CNGs, CuS/Qu–CNGs, and CuS/Qu–CNGs+H_2O_2 groups compared to those of control groups, in the presence of NIR, respectively (***$p < 0.001$).

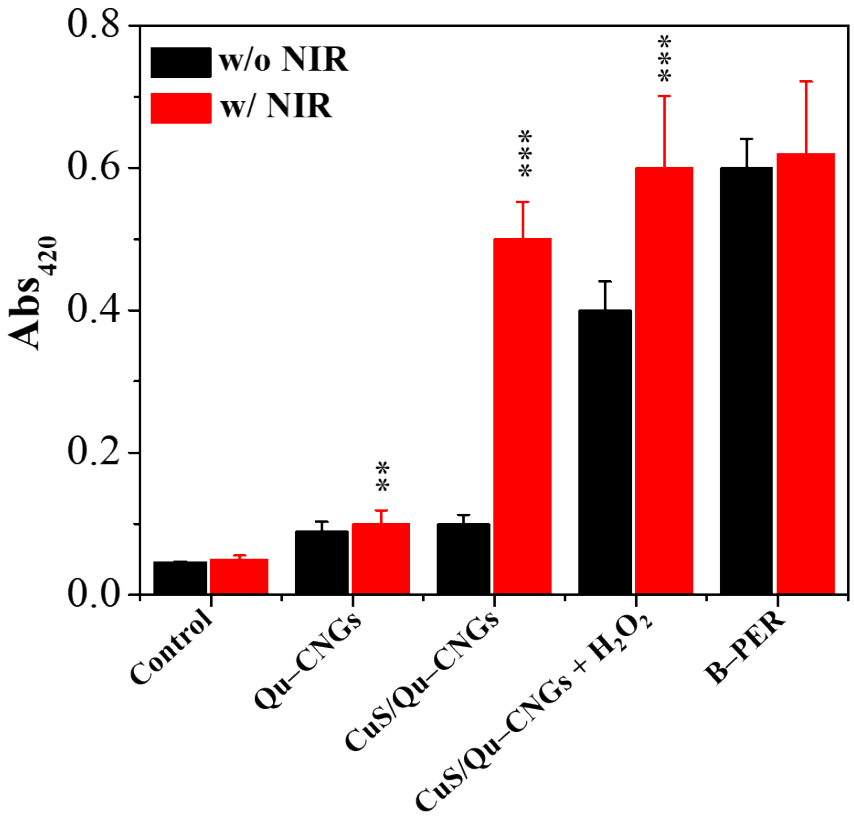

Fig. S15. Absorbance values of *o*-nitrophenol (ONP) at 420 nm (Abs_{420}) from the supernatants of *E. coli* (2.0×10^8 CFU mL^{-1}) solutions untreated and incubated with bacterial lysis solution (B-PER), 0.2 μg mL^{-1} of Qu–CNGs or CuS/Qu–CNGs in the absence and presence of H_2O_2 (10 μM) in 10 mM sodium phosphate buffer (pH 7.4) for 1 h without (w/o) or with (w/) NIR-II irradiation (1064 nm, 0.94 W cm^{-2}, 10 min). The error bars represent the standard deviation of four repeated measurements. Asterisks indicate statistically significant differences of the Qu–CNGs, CuS/Qu–CNGs, and CuS/Qu–CNGs+H_2O_2 groups compared to those of control groups, in the presence of NIR, respectively (**$p < 0.01$ and ***$p < 0.001$).

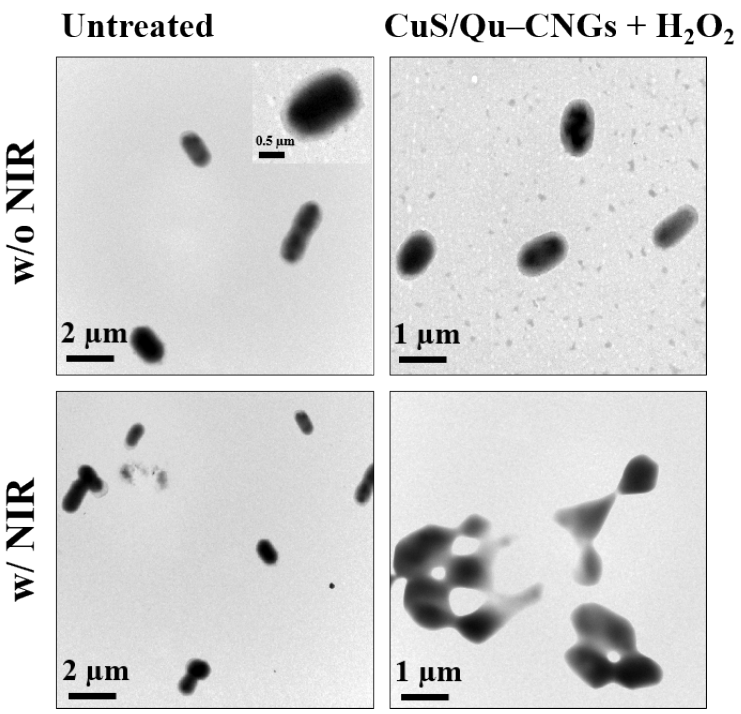

Fig. S16. TEM images of *E. coli* (2.0×10^8 CFU mL^{-1}) untreated and treated separately with 0.2 μg mL^{-1} of CuS/Qu–CNGs in the presence of H_2O_2 (10 μM) in 10 mM sodium phosphate buffer (pH 7.4) for 1 h without (w/o) or with (w/) NIR-II irradiation (1064 nm, 0.94 W cm^{-2}, 10 min).

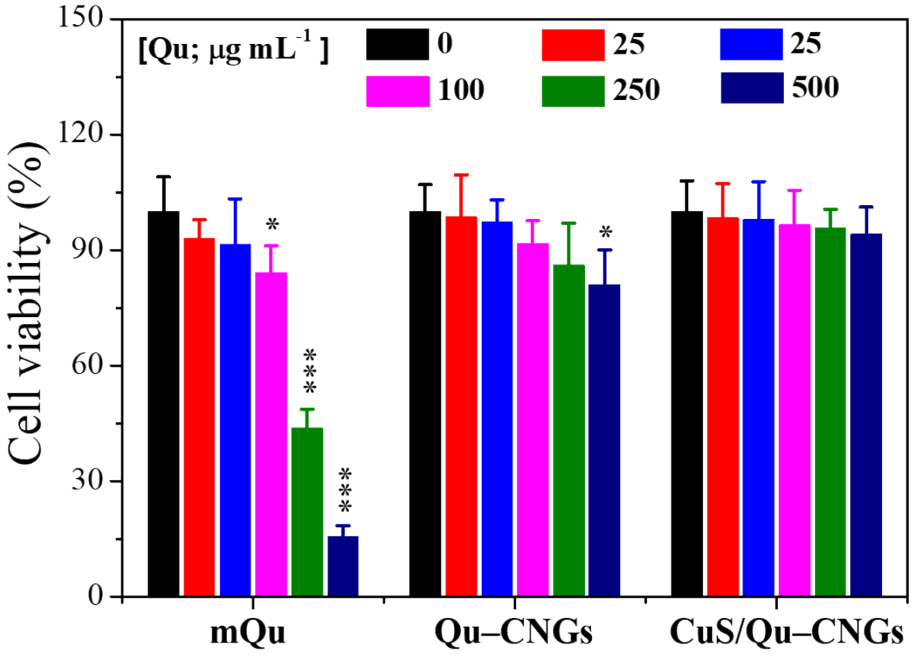

Fig. S17. Relative cell viabilities of HaCaT cells (1.0×10^4 cells well^{-1}) after separate incubation with various concentrations of mQu, Qu–CNGs, and CuS/Qu–CNGs in DMEM containing 10% FBS for 24 h at 37 °C. The error bars represent the standard deviation of four repeated measurements. Asterisks indicate statistically significant differences of the mQu, Qu–CNGs, and CuS/Qu–CNGs groups compared to those of untreated ones, respectively (*$p < 0.05$, ***$p < 0.001$).

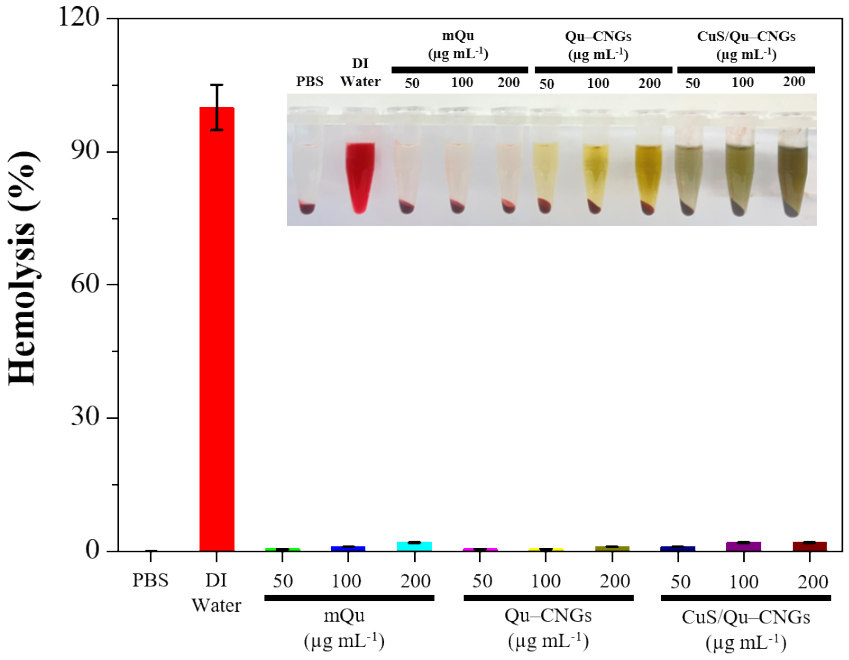

Fig. S18. Hemolytic activities against RBCs (4% v/v) incubated with various concentrations of mQu, Qu–CNGs, or CuS/Qu–CNGs dispersed in PBS. The RBCs treated with PBS and DI water served as negative and positive controls, respectively. Insets: photographs of the corresponding solution. The error bars indicate the standard deviation of three repeated measurements.

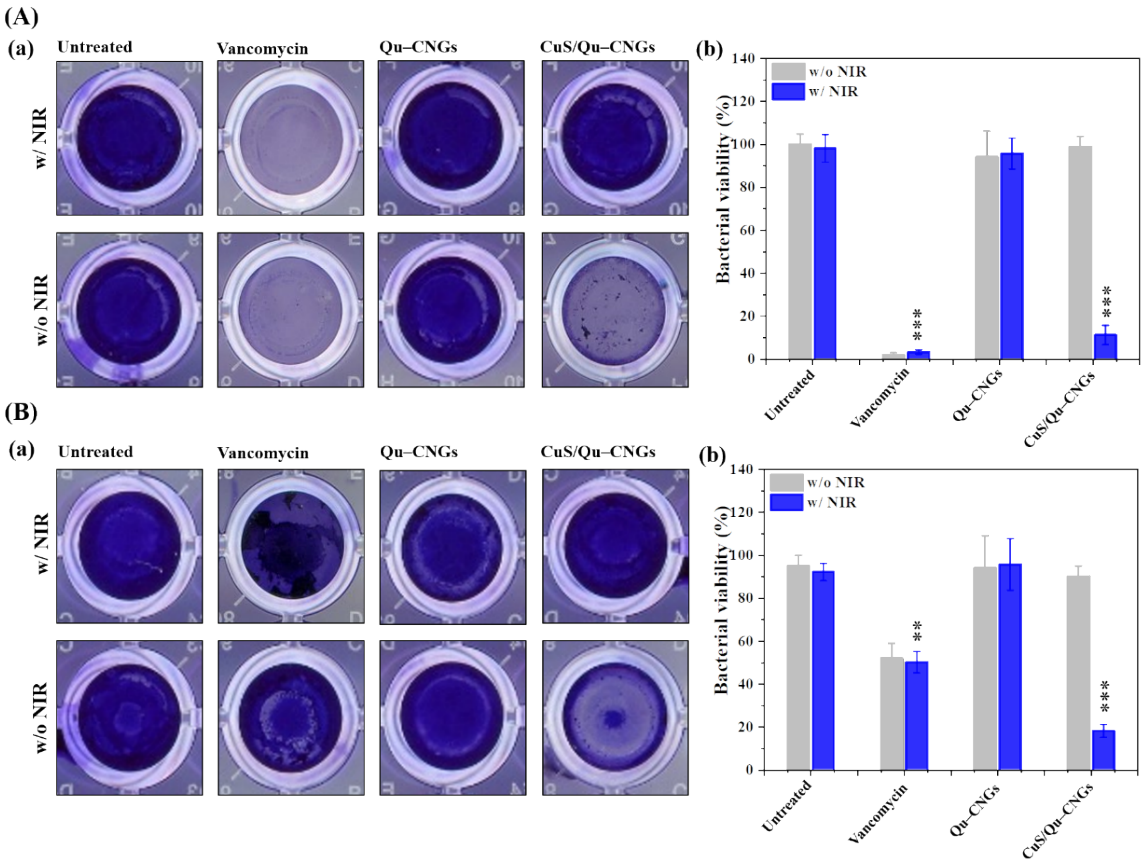

Fig. S19. (A) Biofilm inhibition and (B) biofilm eradication assay of (a) photographies of MRSA untreated and treated with vancomycin (100 μg mL^{-1}) or 1.0/5.0 μg mL^{-1} of Qu–CNGs or CuS/Qu–CNGs without and with NIR-II laser irradiation (1064 nm, 0.94 W cm^{-2}) for 10 min and then stained with crystal violet dye solution (0.1%). (b) The corresponding bacterial biofilm viabilities were determined through the absorbance values of crystal violet staining. The concentrations of Qu–CNGs and CuS/Qu–CNGs (in terms of Qu–CNGs) applied for biofilm inhibition and eradication are 1.0 and 5.0 μg mL^{-1}, respectively. The error bars represent the standard deviation of three repeated measurements. Asterisks indicate statistically significant differences of the vancomycin and CuS/Qu–CNGs treated groups compared to those of untreated ones, in the presence of NIR, respectively (**$p < 0.001$ and ***$p < 0.0001$).

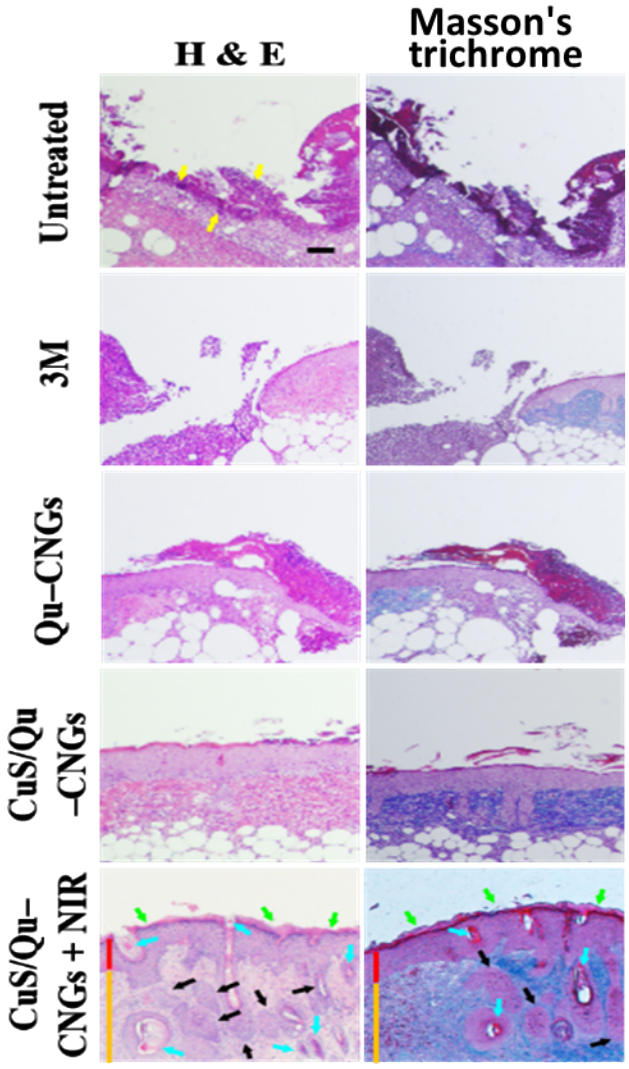

Fig. S20. Histological analysis of tissue sections isolated on day 15 from the wounds untreated and treated with 3M bandage and 25 μg mL^{-1} of Qu–CNGs or CuS/Qu–CNGs (w/o and w/ NIR-II) (1064 nm, 1.32 W cm^{-2}, 10 min). Red and yellow lines represent the thickness of epidermis and dermis, respectively. Scale bar is 500 μm.

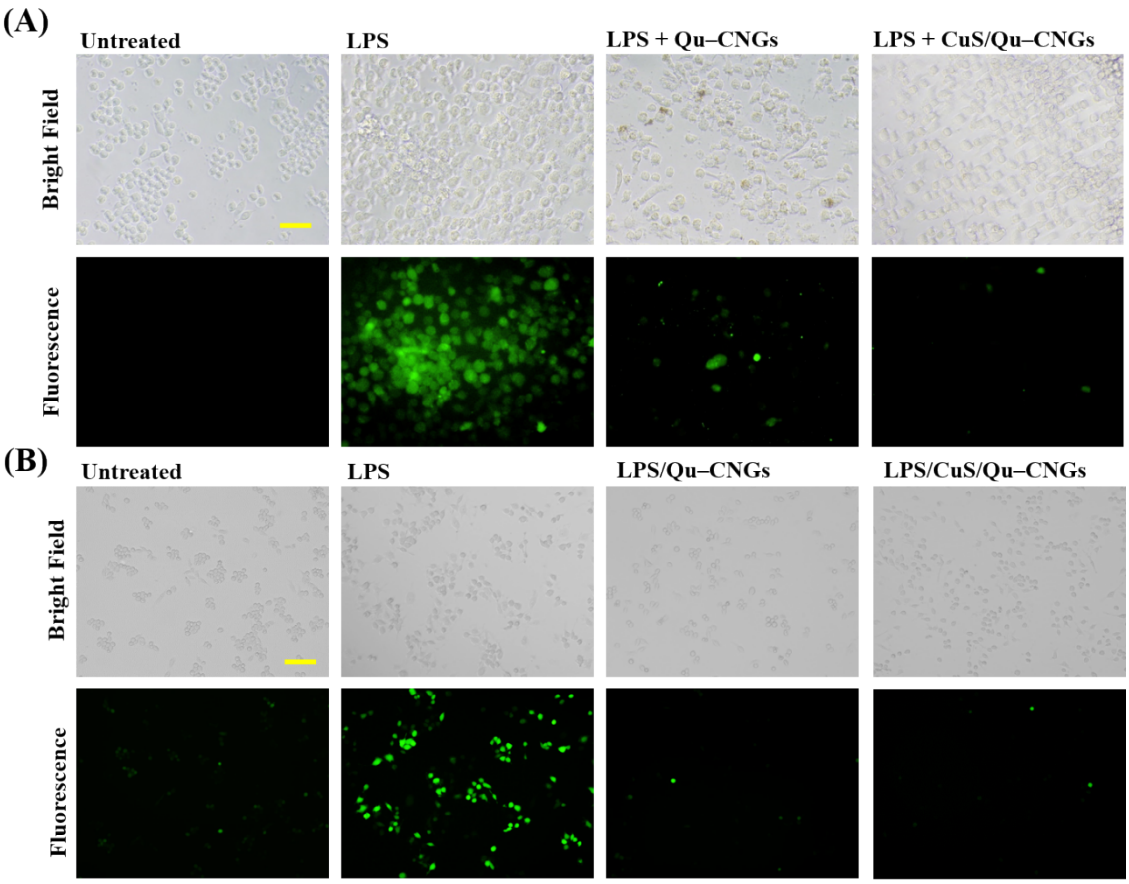

Fig. S21. Bright-field and fluorescence images of RAW264.7 macrophages (1×10^5 cell per well) (A) post and (B) pre-incubated with LPS (1.0 μg mL^{-1}) in the presence of 25 μg $mL^{-1}$of Qu–CNGs or CuS/Qu–CNGs for 18 h in 5% CO_2 at 37 °C and then staining with DCFH-DA for 30 min. The scale bar is 200 μm.

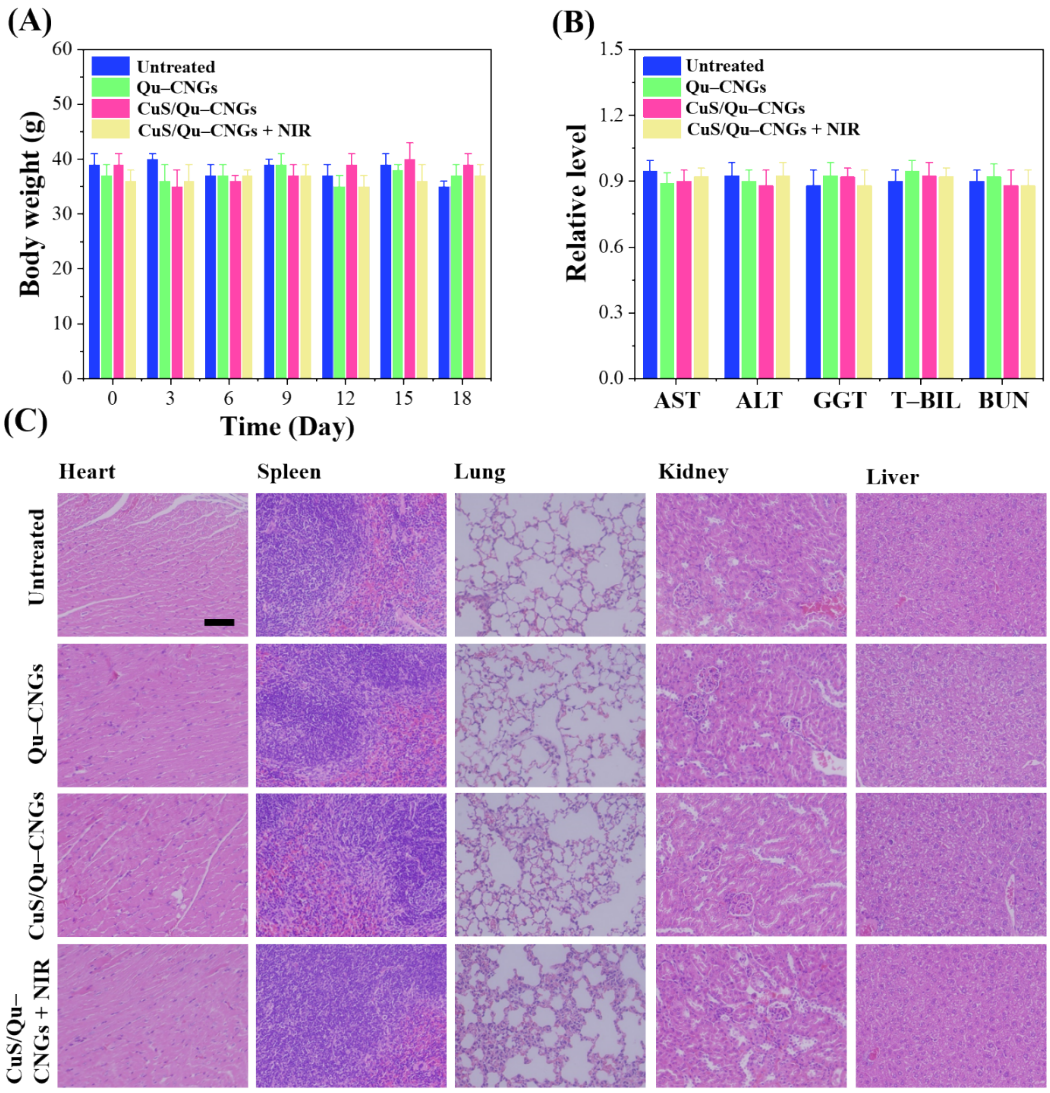

Fig. S22. (A) Body weight of untreated and Qu–CNGs or CuS/Qu–CNGs (w/ and w/o NIR-II) treated mice for 18 days. (B) Relative levels of AST, ALT, GGT, T–BIL, and BUN in the blood stream of mice before and after administration of Qu–CNGs or CuS/Qu–CNGs in the absence and presence of NIR-II for 18 days. (C) H&E staining of the excised tissue sections (thickness~6 μm) on postoperative day 18. Scale bar in (C) is 100 μm. Error bars in (A) and (B) represent the standard deviation of three repeated measurements.