Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Biocompatible Bacteria@Au Composites Assisted for Photothermal Therapy in Cancer Cells

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Experimental Section

XRD and TEM. The crystalline structures were identified using an X-ray diffractometer (Thin-Film XRD, Rigaku D/MAX 2500 Diffractometer) with $Cu_{k\alpha}$ radiation (λ = 1.54060 Å) at 40 kv and 100 mA. Electron micrographs using transmission electron microscopes (JOEL 1200, at 80KV, JEOL 3010, at 300KV and PHILIPS CM-200, at 200KV) were obtained by placing a drop of the sample on a copper mesh coated with an amorphous carbon film, followed by evaporation of the solvent in a vacuum desiccator.

TEM observation of negatively stained bacteria samples. Bacteria were picked up from colonies and suspended in 1% aqueous sodium phosphotungstate solution at pH 7. Droplets of the suspensions were allowed to dry on grids coated with Formvar. The samples were then subject to TEM (JOEL 1200 and JEOL 3010) observation at 80KV and 200KV.

TEM observation of thin sectioned Bacteria. One million bacteria were washed in a 0.1M phosphate buffer at pH 7.2. The cells were precipitated by centrifugation for 3 min at 3,000 rpm. The pallets were fixed in the same buffer with 2% paraformaldehyde and 1.25% glutadehyde for 10 min and then post-fixed in 1% OsO4 aqueous solution for 1 h, washed in distilled deionized H_2O for 10 min 3 times, and dehydrated in a gradient of ethanol and pure propylene oxide (PO). The samples were embedded in Epon resin at room temperature and polymerized in an oven at 55℃ for 1 day. The resin blocks were sectioned into 80 nm thin film and collected onto the copper grids. Sections were stained with lead citrate and uranyl acetate. Observations were performed using TEM.

THG imaging of *E. coli*. In this investigation, a femtosecond Cr:forsterite laser with a wavelength of 1230 nm and a repetition rate of 110 MHz was used to generate THG signals (410nm). The collimated laser beam was guided into an Olympus FV300 scanner connected with an Olympus IX71 inverted microscope. After passing through a pair of galvanometer mirrors in the scanner and the tube lens of the microscope, the 2D-scanning laser beam was finally focused onto the sample by a high-NA (NA 0.9) water-immersion objective. The average power after the objective was about 100 mW. Under this condition, the lateral resolution reached about 400 nm. The morphology of the *E. coli* could be clearly observed by THG.

Cell culture. Human keratinocyte nonmalignant cell line (HaCaT) and human lung carcinoma malignant cell line (A549) were cultured in Dulbecco's modification of Eagle's medium (DMEM, Cellgro) plus 10% fetal bovine serum (FBS, Gem Cell) at 37℃ under 5% CO_2 in air. The cells were collected by trypsinization and placed onto a 10 cm tissue culture Petri dish, then allowed to grow for 2-3 days.

Cytotoxicity assay (WST-1). 5×10^3 cells per well were seeded in a 96-well culture

plate and incubated overnight. Different concentrations of the *E. coli* (DH5α) and DH5α@Au composites were added to the medium, which was then used to replace the original culture medium. Incubation was continued for an additional 24 h. The culture medium was removed and replaced with the new culture containing WST-1 reagents (10%). The cells were then incubated for 1.5 h to allow formation of the formazan dye at 37℃. The reaction product was transferred to a new ELISA plate and A_{450} was measured with a ELISA plate reader (Varioskan, Thermo Electron). The cell growth activity as a percentage of the untreated control was then calculated.

Anti-EGFR antibody conjugation and cellular incubation with DH5α@Au-Ab$_{EGFR}$ composites (DH5α@Au conjugated with anti-EGFR). The anti-EGFR antibody (1mg/ml) (R&D) was mixed with DH5α@Au composites with a volume ratio of $V_{antibody}/V_{DH5\alpha@Au\ composites}$=1/1 for incubation of 40 min at 4℃. They were centrifuged at 12000 rpm for 15 min at 4 ℃ and the supernatant was then discarded. Pellets were rinsed and suspended with 40 mM of HEPES solution. The cell monolayer with A549 and HaCaT was immersed with DH5α@Au-Ab$_{EGFR}$ composites for 24 h, respectively.

Photothermal therapy. A CW diode laser at 808 nm was selected for the laser irradiation experiments (ENWAVE OPERONICS. INC., MODEL FSL-808-450MS-FS). This wavelength, overlapping with the absorption band of the DH5α@Au composites, is in the NIR region at which the tissue has low absorption. The cells (A549 and HaCaT) were immersed into the DH5α@Au-Ab$_{EGFR}$ composites (100μg mL^{-1}) for 24 h and rinsed with PBS buffer. Subsequently, laser exposure at various power densities (24 to 30 W cm^{-2} irradiation) was performed for 6 min. The laser was focused to a 1 mm diameter spot on the sample. Cells were then stained with an organic fluorescent dye, calcein AM, to examine cell viability. Staining cells were observed using a NIKON ECLIPSE TS100 microscope using EVOLUTION VF CCD and IMAGE-PRO PLUS Ver 6.2 software.

Temperature dependence of irradiation time for DH5α@Au composites at 808nm laser irradiation. The temperature elevation of the DH5α@Au composites obtained from methods 1 and 2 were measured by placing the composite solutions (100uL, 100μg mL^{-1}) in 96-well plates using a 808nm CW diode laser in a range of irradiation times (1-8min). A thermalcouple (not exposed to the laser beam) was immersed in the composite solutions to determine temperature. The CW laser had a beam spot close to 1 mm^2 and a power output of 260mW (26W cm^{-2}). The temperature in solution was determined by thermalmeter (TES 1319A–K type).

Table.S1 Genotypes of the three different strains of *E. coli* were chosen in this study.

Strain	Genotype
DH5 α	*F'/endA1 hsdR17($r_k^-m_k^+$) supE44 thi-1 recA1 gyrA (Nal^r) relA1 △(lacIZYA-argF)U169 deoR (ϕ80dlac △(lacZ)M15)*
TOPO 10	*F-mcrA△(mrr-hsdRMS-mcrBC) ϕ80lacZ△M15 △lacX74 deoR recA1 araD139△(araA-leu)7697 galU galK rpsL endA1 nupG*
JM 109	*F' tra36 lac^q △(lacZ)M15 $proA^+B^+$/e14-(McrA-) △(lac-proAB) recA1 thi gyrA96(Nal^r) endA1 hsdR17($r_k^-m_k^+$) relA1 supE44*

Table.S2 Average lengths and widths of *E. coli* bacteria.

Strain	Length (μm)	Width (μm)
DH5 α	2.39± 0.77	0.69± 0.24
TOPO10	2.13± 0.64	0.87± 0.31
JM109	2.08± 0.58	0.58± 0.21

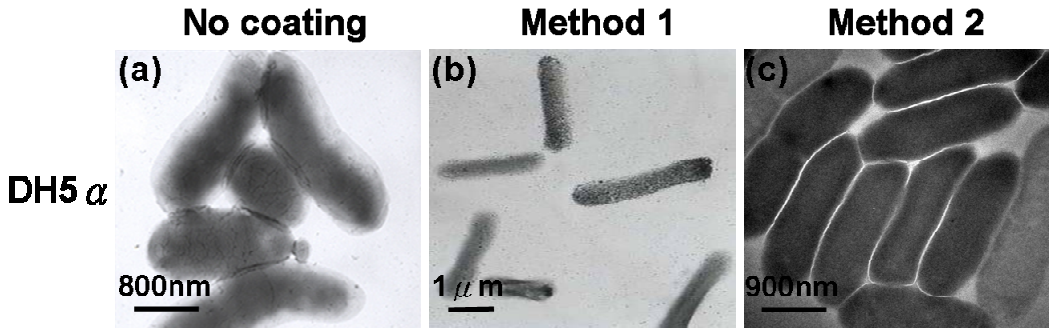

Fig.S1 Bacteria with or without multi gold nanoshells were imaged by TEM. (a) Showing bacteria without gold NPs, where bacteria with negative staining using 1% aqueous sodium phosphotungstate solution. (b) By method 1 and (c) by method 2 showing gold NPs formation for *E.coli*, DH5α

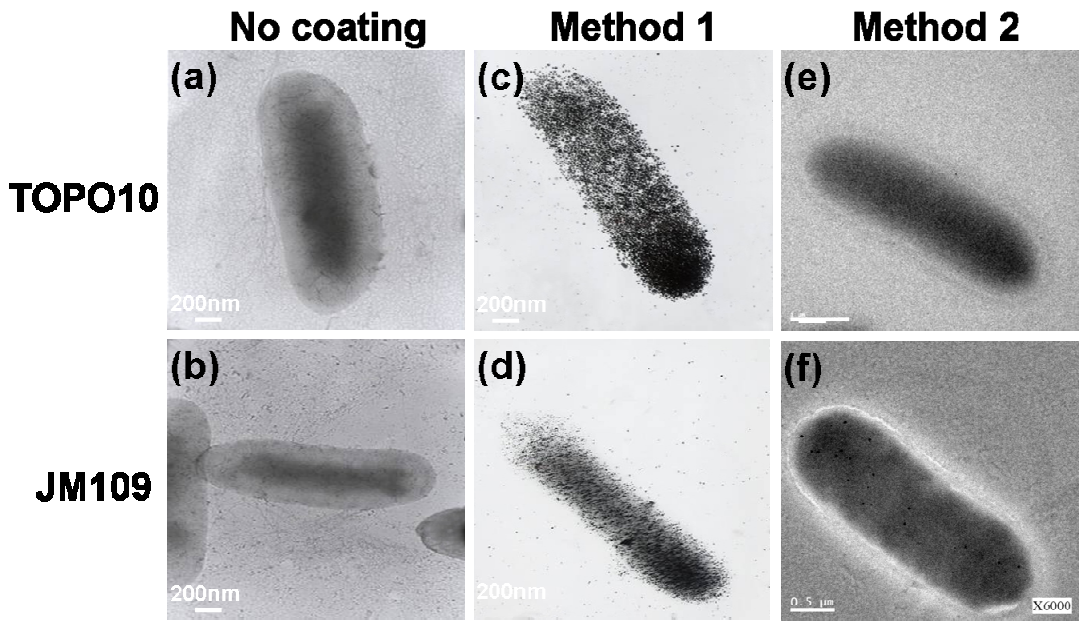

Fig.S2 Bacteria of *E. coli*, TOPO10 and JM109, with or without gold nanoshells were imaged by TEM. (a-b) Showing bacteria without gold NPs. (c-d) By method 1 and (e-f) by method 2 showing gold NPs formation.

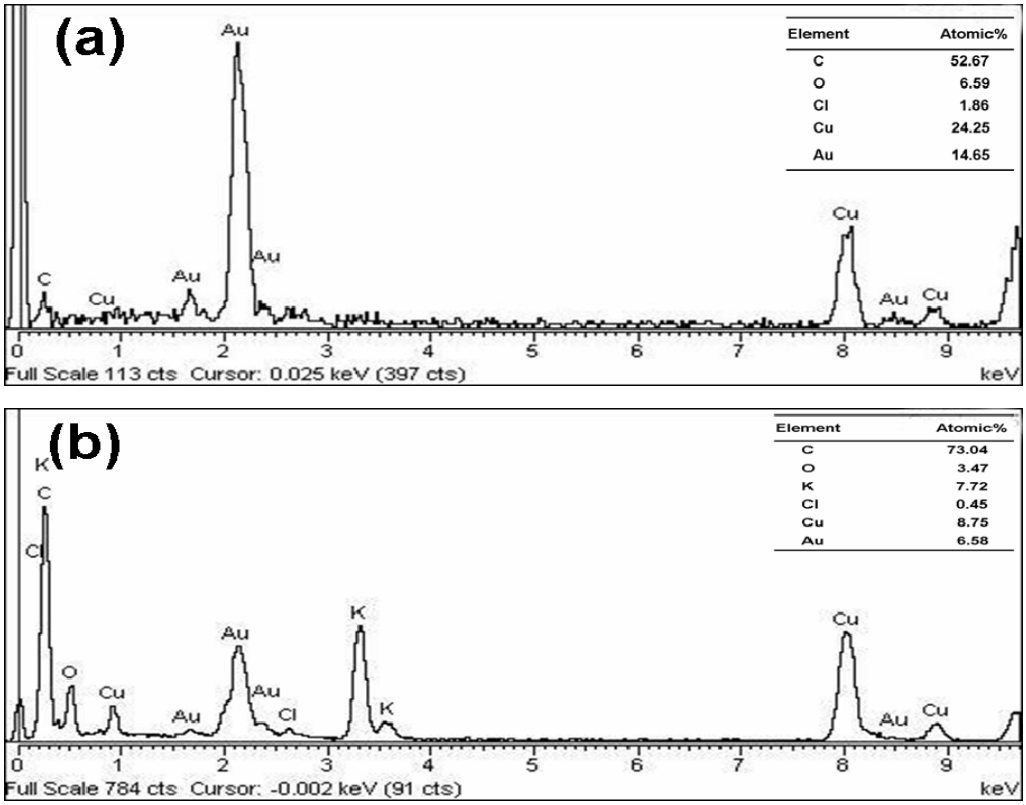

Fig.S3 EDX analysis of DH5α@Au composites derived from (a) method 1 and (b) method 2.

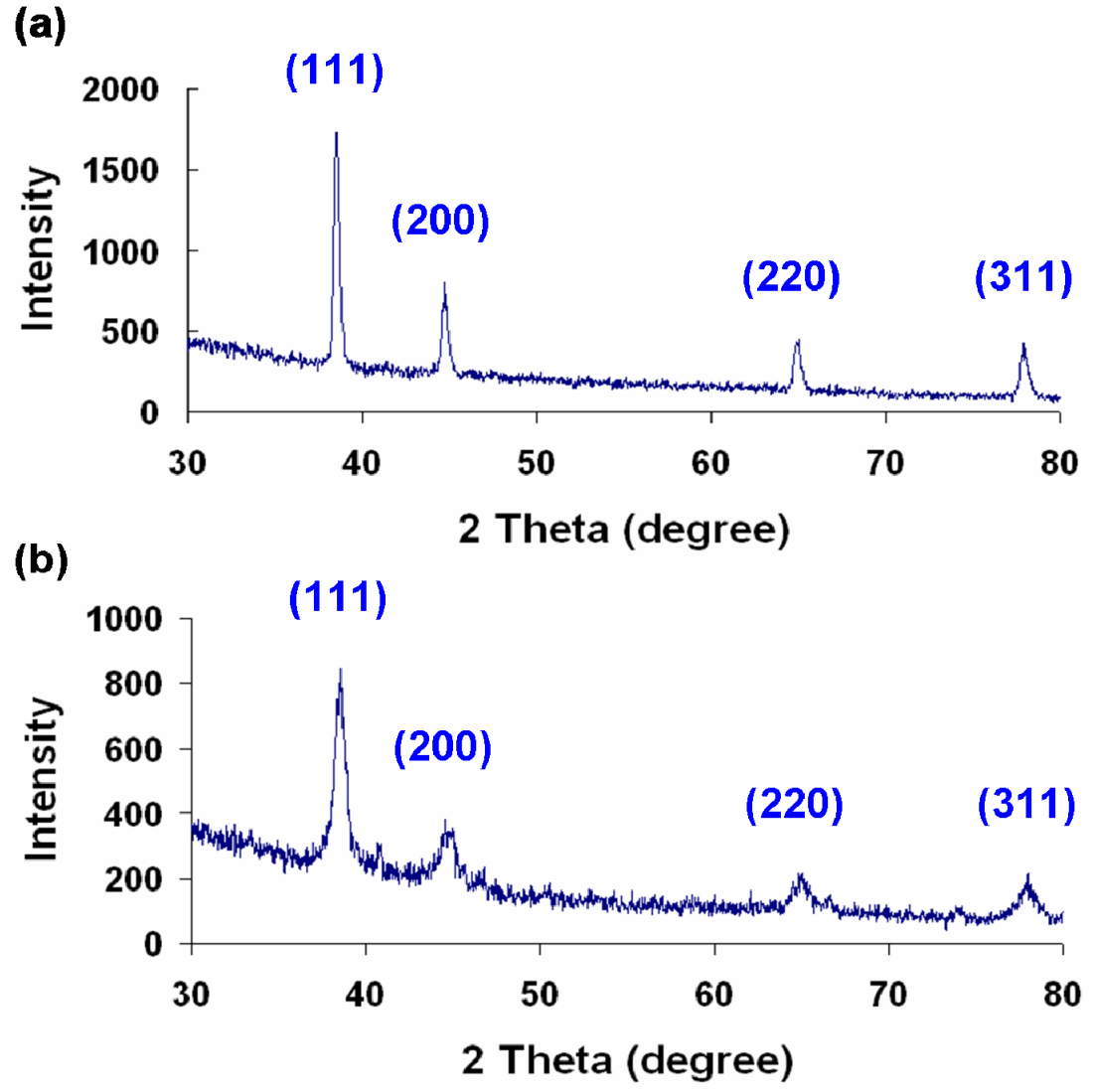

Fig.S4 XRD patterns of DH5α@Au composites derived from (a) method 1 and (b) method 2.

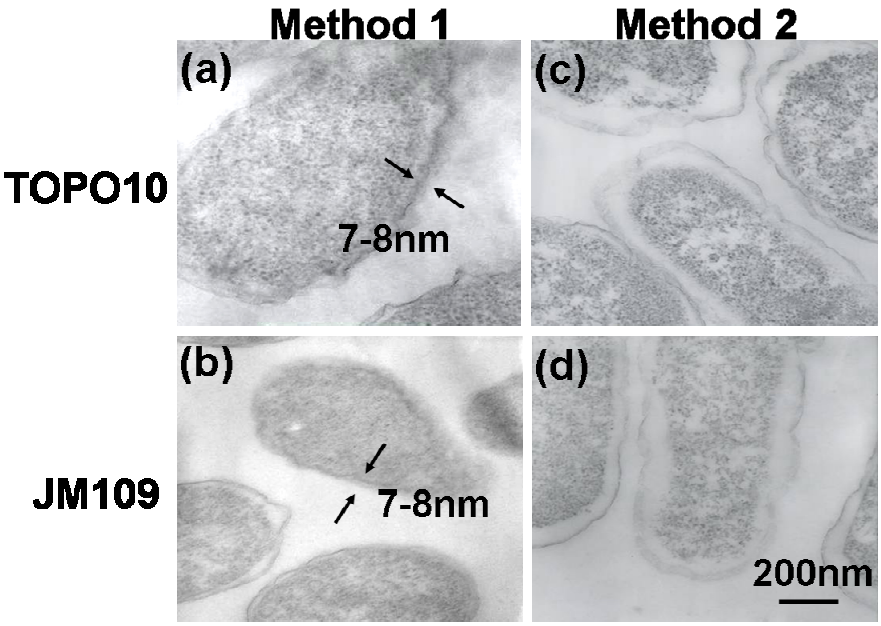

Fig.S5 Ultramicrotomed images for bacteria, TOPO10 and JM109, coated with gold nanoshell (a-b) by method 1 and (c-d) by method 2. The gold nanoshells generated by both methods were approximately 7-8 nm thickness after 32h incubation of metal precursors with bacteria.

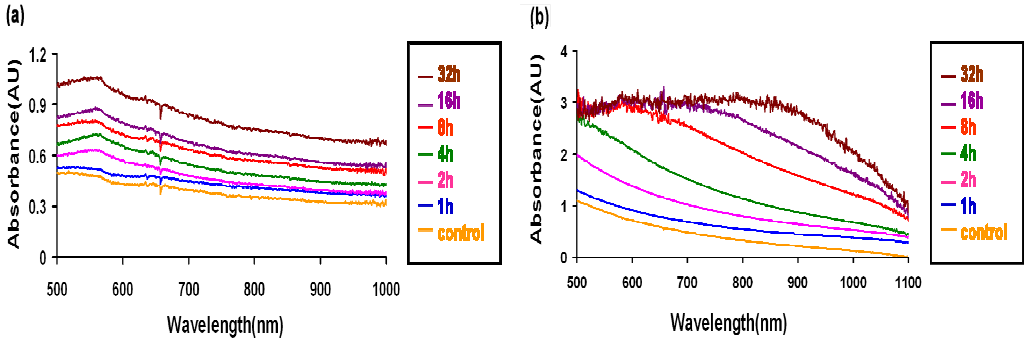

Fig.S6 UV-visible spectra of DH5α@Au composites as a function of incubation time (1-32 h) for (a) method 1 and (b) method 2.

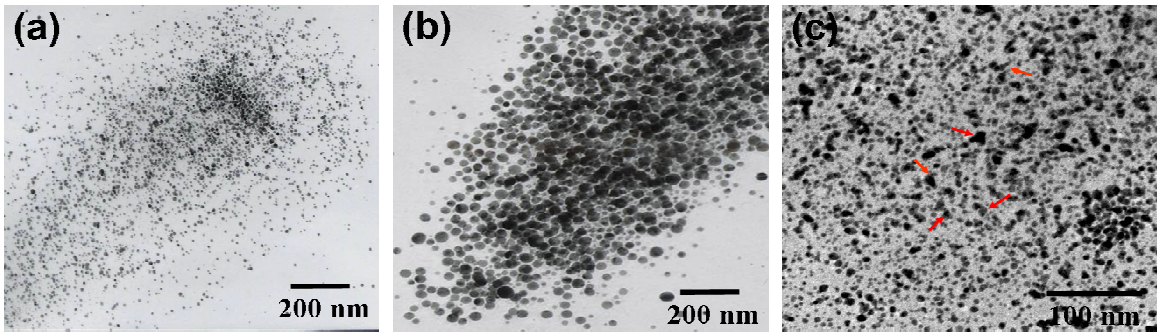

Fig.S7 TEM images showing DH5α@Au composites obtained from method 1 after incubation of (a) 1 h and (b) 32 h and from method 2 after incubation of (c) 32 h. The red arrows in (c) indicate rod-like and triangle-like particles.

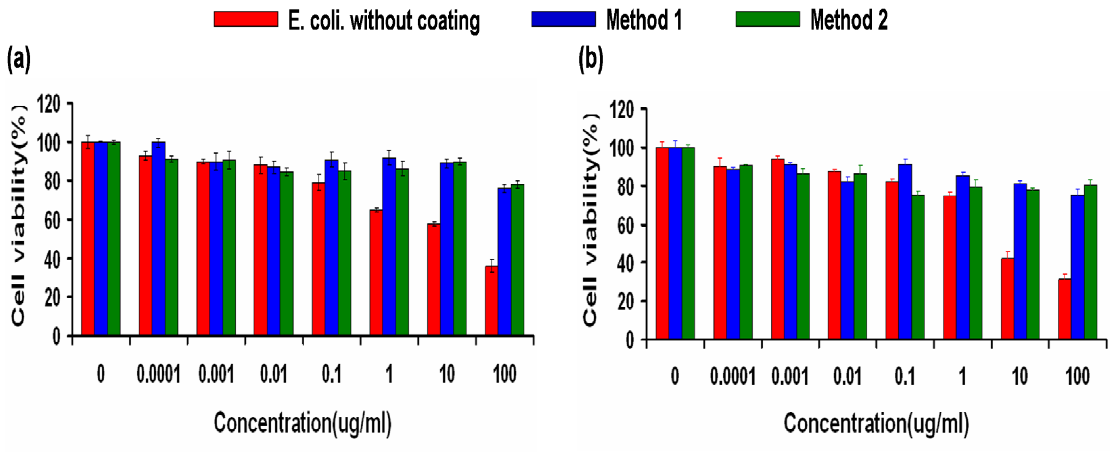

Fig.S8 The biocompatibilities of the *E. coli* (DH5α) alone and bacteria@Au composites derived from method 1 and method 2 were analyzed using WST-1 assay. (a) HaCaT and (b) A549 cells were incubated with samples (*E. coli* alone and bacteria@Au composites) for 24 h.

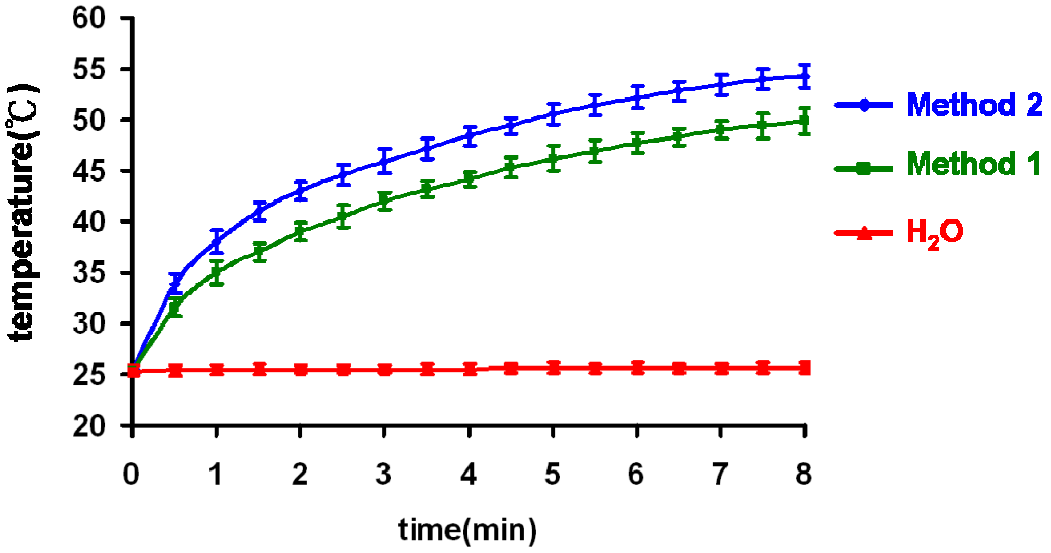

Fig.S9 The temperature dependence of NIR-irradiated H_2O, DH5α@Au composites (method 1 and 2) as a function of irradiation time at a power density of 26W cm^{-2}.

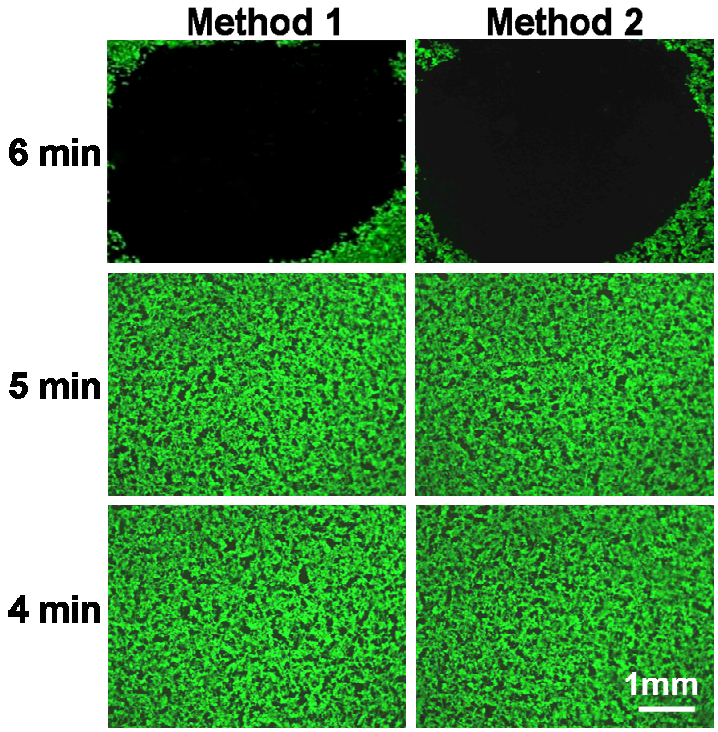

Fig.S10 Anti-EGFR antibody conjugated with DH5α@Au composites (100 μg mL^{-1}) of (a) method 1 and (b) method 2 treated with A549 cancer cells were irradiated by laser dosages of 260mW (26W cm^{-2}) for 4-6 min. The column showed staining by calcein AM as green fluorescence dye for living cells.