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Multifunctional hybrid materials for combined photo and chemotherapy of cancer

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Supporting Information

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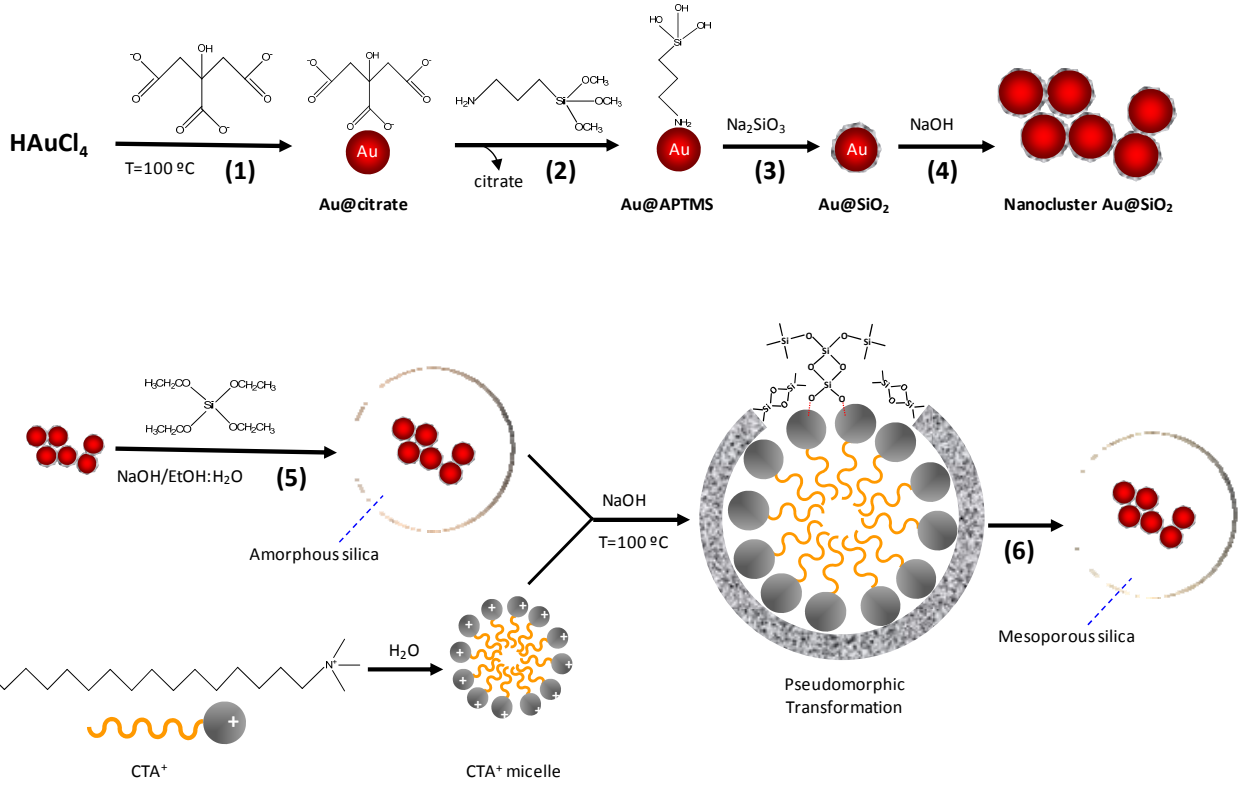
Synthesis and characterization of materials

Synthetic process



Scheme S1 Schematic representation of the different steps in the synthesis process of mesoporous silica protected gold nanoclusters. (1) Stabilizing GNP with citrate. (2) Activation of GNP surface with APTMS. (3) Development of a homogeneous, thin (1-2 nm) silica coating on GNP. (4) Controlled aggregation of GNP into nanoclusters in alkaline medium. (5) Growth of silica wall over single GNP by hydrolysis of TEOS according to Stober's method. (6) Pseudomorphic transformation of the silica wall in an alkaline solution of cetyltrimethyl ammonium (CTA^+) bromide.

TEM study

Table S1 Gold content and nanoclusters distribution in mesoporous silica-protected gold nanoclusters.

Au (wt%)[a]	Clusters distribution[b]			
	0	1-4	5-9	≥10
18.9	14	26	44	16

[a] As determined by ICP in the calcined hybrid nanoparticles.

[b] Relative abundance of gold nanoparticles in as-prepared clusters (NaOH=4 mM). Estimation from TEM images by measuring at least 200 particles.

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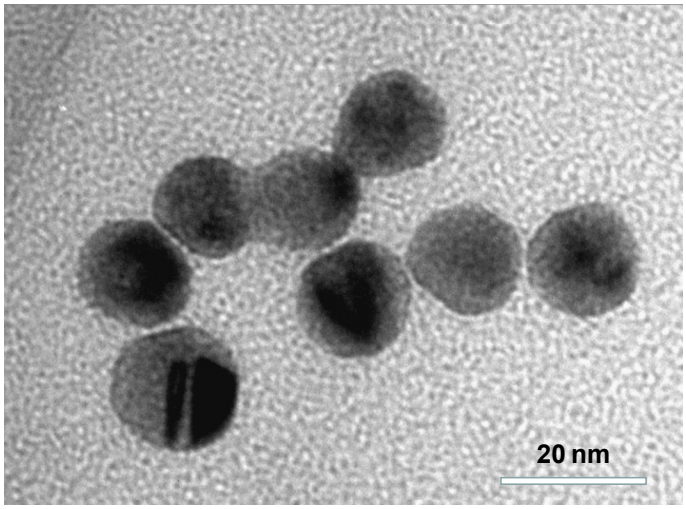

Fig. S1 Colloidal gold nanoparticles (GNP) as synthesized by standard citrate reduction method.

X-Ray diffraction and nitrogen adsorption-desorption isotherms

Table S1 Textural and structural characteristics of mesoporous silica-protected gold nanoclusters.

Sample	Vp (cm^3/g)[a]	S_{BET} (m^2/g)[b]	D (Å)[c]	d_{100} (Å)[d]	w_d (Å)[e]	b_d (Å)[f]
Nanoclusters $Au@SiO_2$	0.49	528	37	40.2	35.1	13.0
Nanoclusters $Au@SiO_2$ + CPT (0.8 wt%)	0.26	449	34	38.6	30.4	15.6

[a] Pore volume of the mesoporous silica wall as determined by nitrogen adsorption data. Value corrected for the pure silica sample.

[b] Surface area of the mesoporous silica wall as determined by nitrogen adsorption data. Value corrected for the pure silica sample.

[c] Pore diameter of the mesoporous silica wall. Calculated by the Kruk-Jaroniec-Sayari method.

[d] XRD (100) interplanar spacing.

[e] Primary mesopore diameter. Calculated from the equation based on geometrical considerations of the ordered honeycomb structure with hexagonal pores.

[f] Pore wall thickness. Calculated under assumption of hexagonal pore geometry.

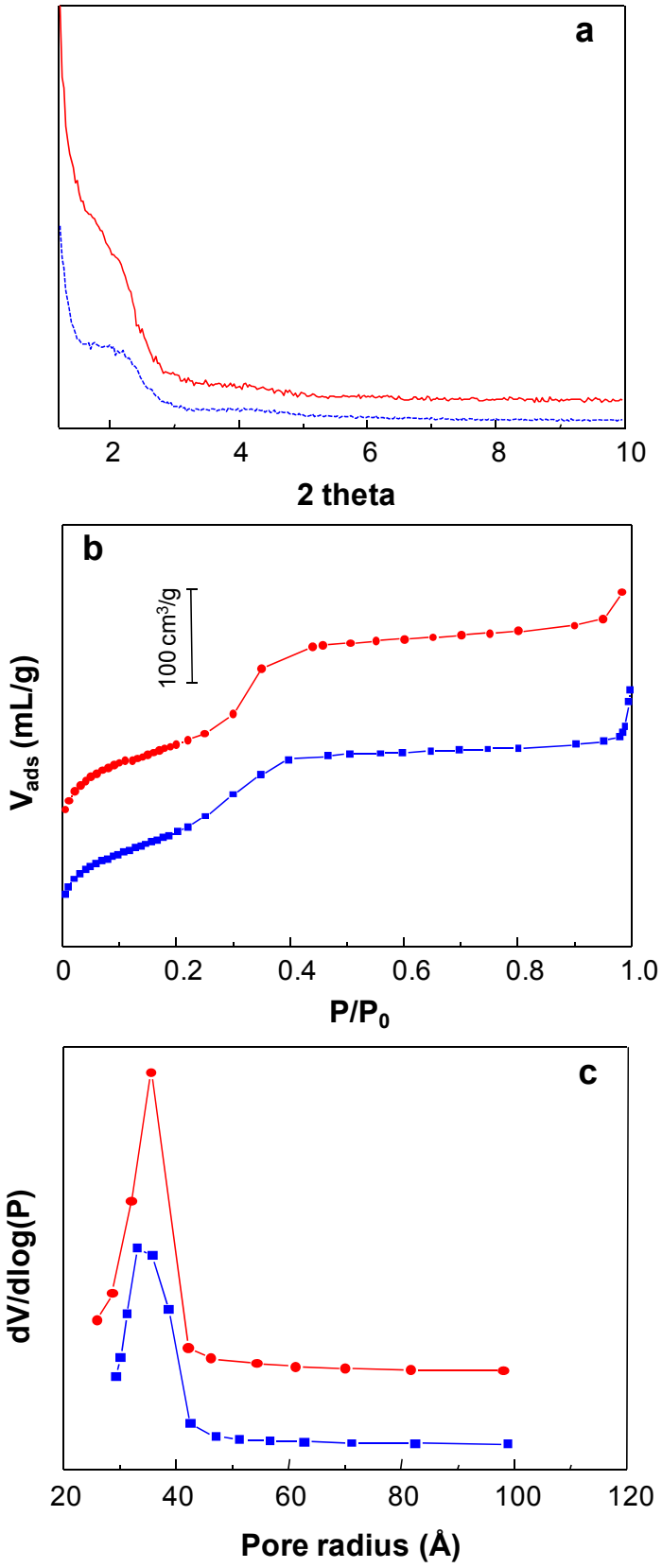

Fig. S2 (a) XRD patterns of the mesoporous silica coating used for protection of gold nanoclusters in the calcined hybrid material before (solid line) and after (dashed line) CPT incorporation. (b,c) BET nitrogen adsorption isotherms (b) and pore size distribution (c) of the mesoporous silica coating before (●) and after (■) CPT incorporation.

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Release of CPT

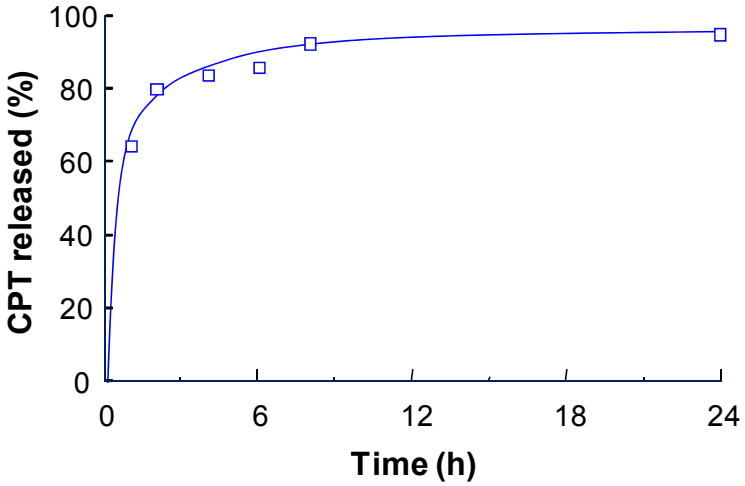

Fig. S3 Release profile of CPT-containing material (0.8 wt%) in PBS at 37 ºC.

Cytotoxicity assay

MTT assay and flow cytometry

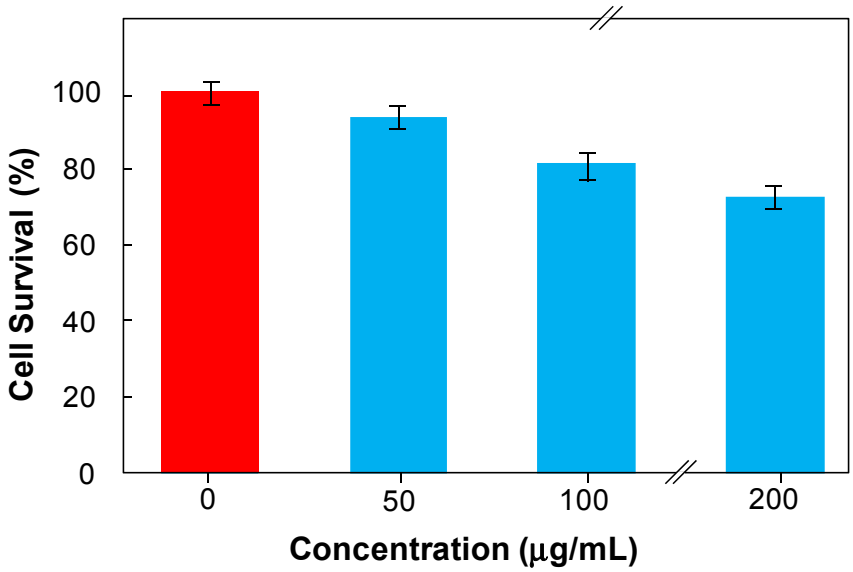

Fig. S4 Percentage of cell viability in MTT test for 42-MG-BA human glioma cells after 24 h incubation with variable concentration of mesoporous silica-protected gold nanoclusters. Each value represents the mean ± standard deviation (μg/mL) of six independent experiments.

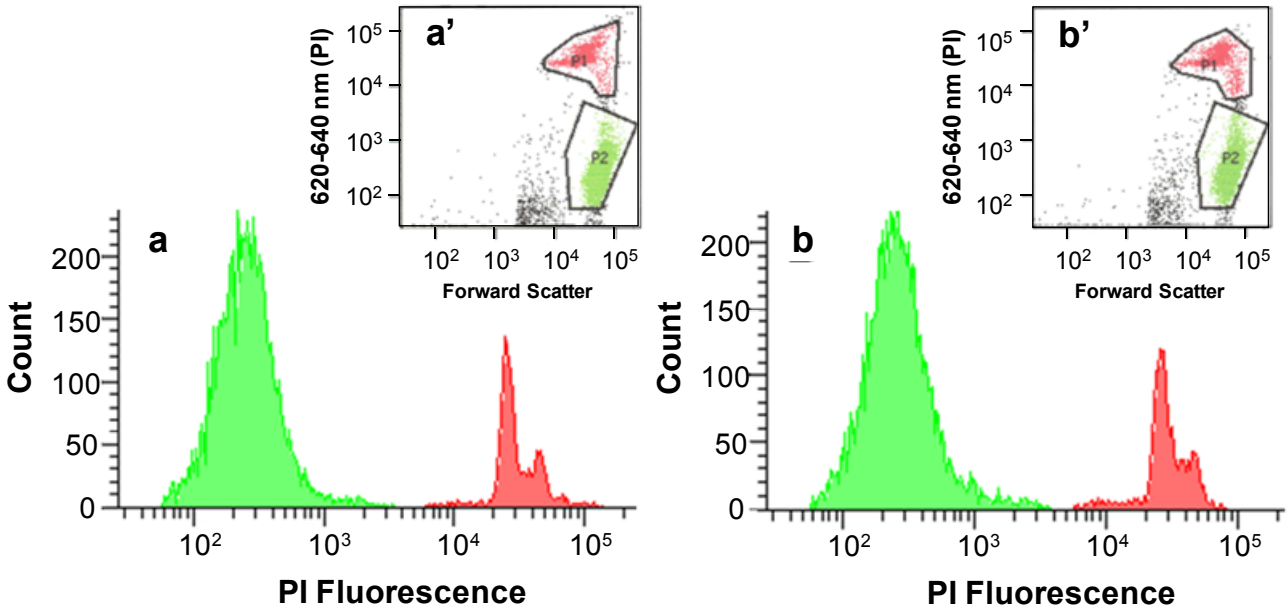

Fig. S5 Flow cytometry graphs analyzed to determine the percentage of cellular death after 42-MG-BA human glioma cells were incubated 24 h with 100 μg/mL mesoporous silica-protected gold nanoclusters. Experiments were performed by using triplicates.

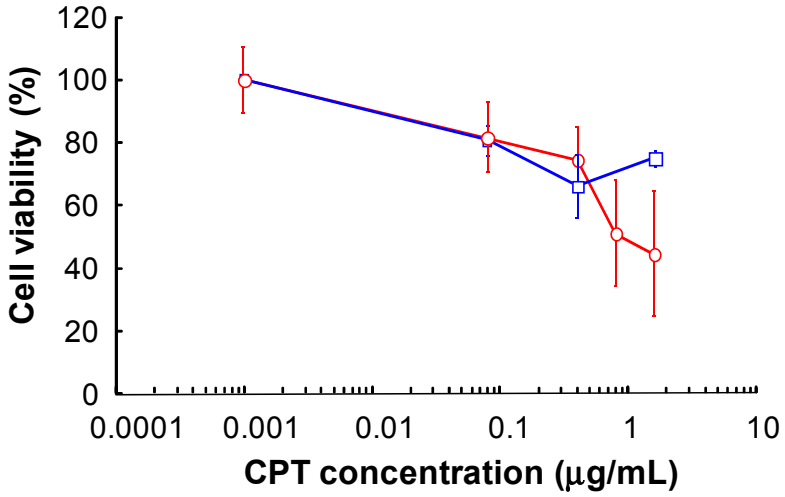

Fig. S6 Cell viability in MTT test for 42-MG-BA human glioma cells after 24 h incubation with variable concentration of CPT free (□) or loaded in mesoporous silica-protected gold nanoclusters (0.8 wt%) (○). Each value represents the mean ± standard deviation (μg/mL) of three independent experiments.

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Laser parameters

Irradiation time

Plates with 240.000 cells/mL 42-MG-BA human glioma cells were seeded and incubated at 37 ºC in a humidified atmosphere containing 5% CO_2 in air for 15 hours until fully confluent. After 24 hours in culture, samples were irradiated with a Ti:Saphire laser system (790 ± 7 nm, 600 mW output power, single pulse, 1.00 ± 0.01 mm spot size, 15-1440 s irradiation time). Immediately after NIR-light exposure, cells were stained and fixed as reported (see Methods).

Results (Fig. S6) show that as the exposure time increased beyond 45 s a significant decrease of the cell density took place. Conversely, little or no damage was observed between samples irradiated up to 45 s ($p>0.05$, $n=5$).

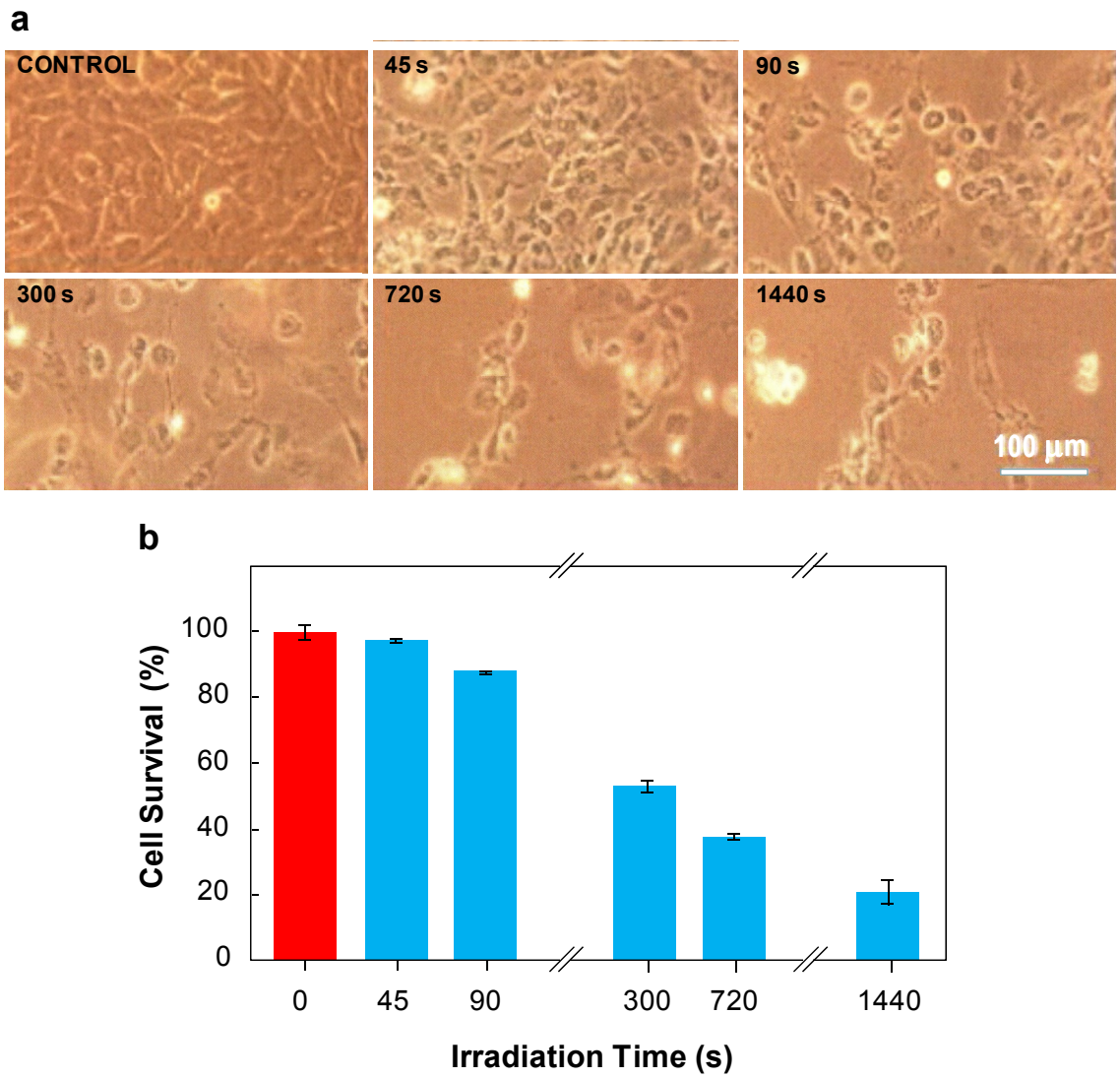

Fig. S7 Effect of laser exposure (P=76 W/cm^2) at different irradiation times on 42-MG-BA human glioma cells. (a) Inverted microscope images; (b) Percentage of cell viability. Control: t=0 s. Each value represents the mean ± standard deviation (µg/mL) of cell counts performed manually in five randomly selected microscopic fields.

Laser power

Plates with 240.000 cells/mL 42-MG-BA human glioma cells were seeded and incubated at 37 ºC in a humidified atmosphere containing 5% CO_2 in air for 15 hours until fully confluent. Then, mesoporous silica-protected gold nanoclusters ($D_{cluster}$=72.8 ± 6.5 nm, $D_{particle}$=158 ± 33 nm) were added to cells up to 100 µg/mL. After 24 hours in culture, samples were irradiated with a Ti:Saphire laser system (790 ± 7 nm, 90-600 mW output power, single pulse, 1.00 ± 0.01 mm spot size, 45 s irradiation time). Immediately after NIR-light exposure, cells were stained and fixed as reported (see Methods).

Results (Fig. S7) stress the need of an energy threshold in order to achieve a significant reduction of cell density. As the power density increased from 90 mW (11 W/cm^2) to 600 mW (76 W/cm^2) the damage for cells treated with gold nanoclusters increases almost linearly (r^2=0.9717), similar to that found by other authors.[47] Cellular photothermolysis reached to 37% for maximum laser power (p>0.05, n=5).

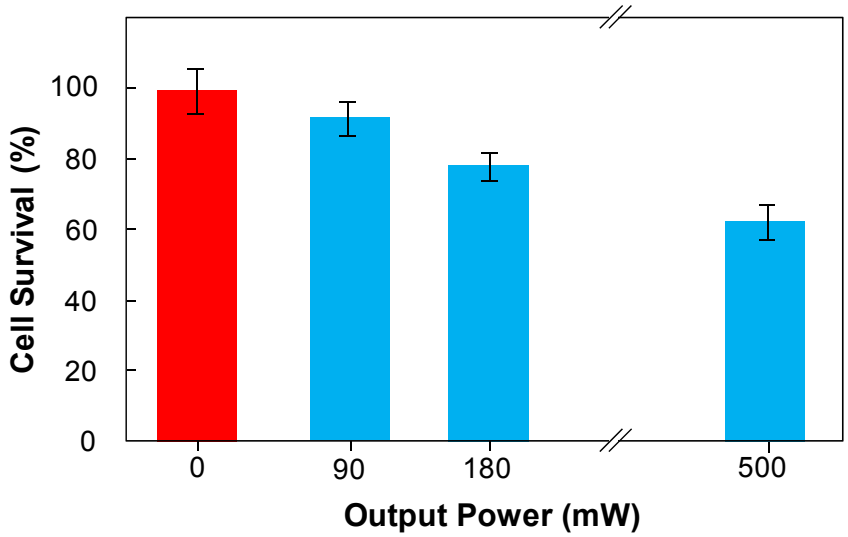

Fig. S8 Graph of cellular damage *versus* laser power density for 42-MG-BA human glioma cells after 24 h incubation with 100 µg/mL mesoporous silica-protected gold nanoclusters. Each value represents the mean ± standard deviation (µg/mL) of cell counts performed manually in five randomly selected microscopic fields.