Electronic Supplementary Material (ESI) for Chemical Communications


# Supporting Information

## Graphene Oxide/Photosensitizer Conjugate as a Redox-Responsive Theranostic

Youngnam Cho[1,2] and Yongdoo Choi[1]*

[1]Molecular Imaging & Therapy Branch, National Cancer Center, 111 Jungbalsan-ro, Ilsandong-gu, Goyang, Gyeonggi-do 410-769, South Korea

[2]Center for Paralysis Research, Department of Basic Medical Science, School of Veterinary Medicine, Purdue University, W. Lafayette, IN 47907, U.S.A.

*Corresponding author: Yongdoo Choi, PhD

Tel.: +82-31-920-2512

Fax: +82-31-920-2529

E-mail: ydchoi@ncc.re.kr

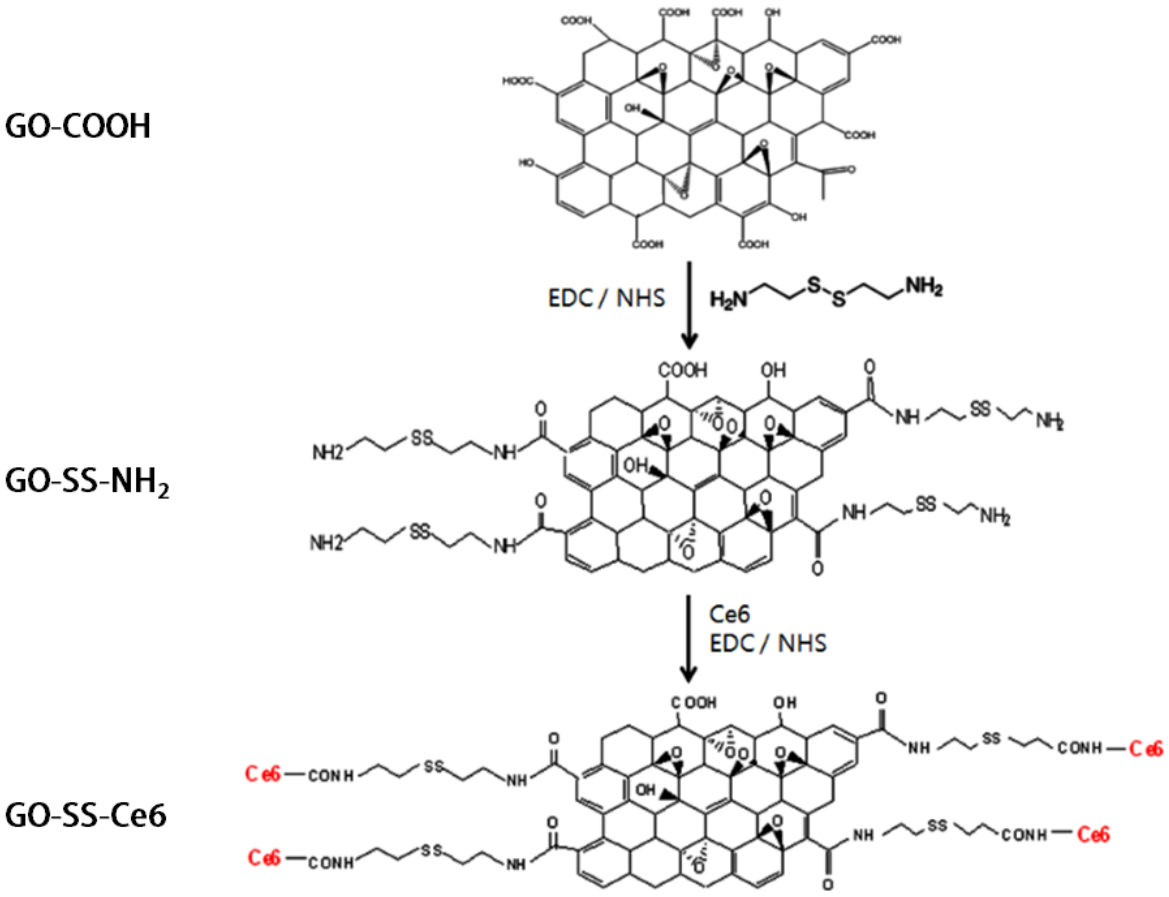


**Figure S1**. Synthetic scheme of GO-SS-Ce6 conjugate.

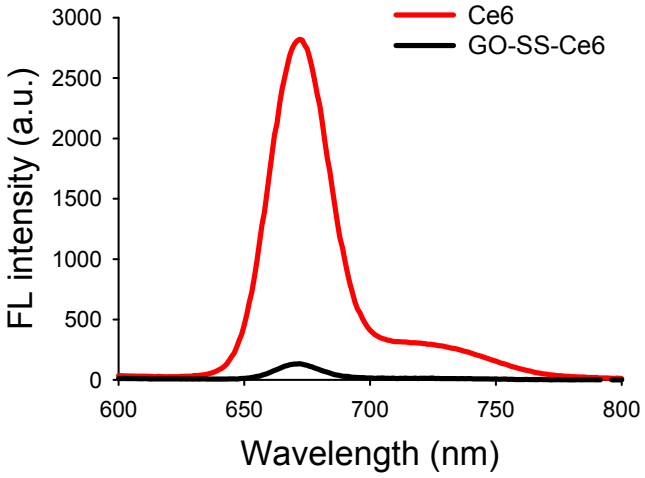


**Figure S2.** Fluorescence intensity spectra of Ce6 and GO-SS-Ce6 at 0.5 μM of Ce6 equivalent.

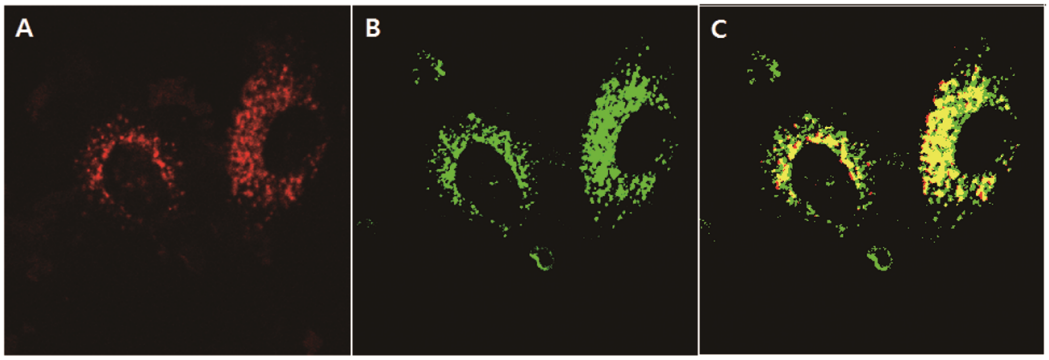


**Figure S3.** (A) ~ (C) Confocal fluorescence microscopy images of A549 cells. Cells were incubated with GO-SS-Ce6 (1.5 μM Ce6 equiv.) for 24 h (magnification 80×). (A) and (B) show fluorescence images stained with red Ce6 and green lysotracker, and (C) shows the merged image.

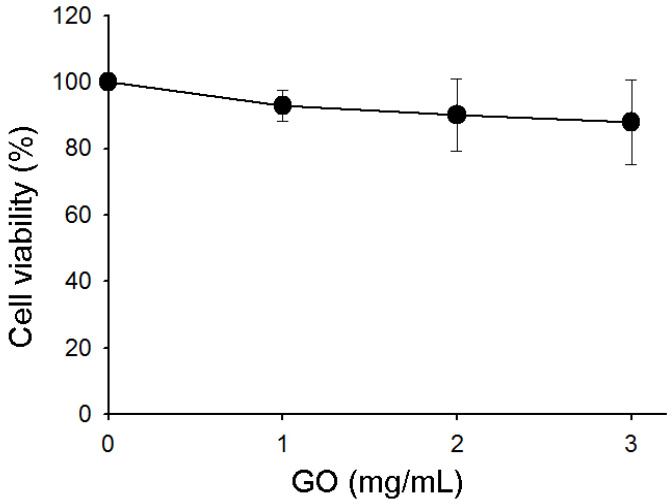


**Figure S4.** Cell viability of A549 cells treated with GO-COOH for 24 h at various concentration.

## Experimental Section

### Preparation of Graphene Oxide-Ce6 Conjugate, GO-SS-Ce6

The concentrated graphene oxide aqueous solution was purchased from Graphene Laboratories Inc. (Calverton, NY, USA). The GO suspension (1 mg/ mL) was sonicated in an ice bath with an ultrasonic probe at 100 W, followed by bath-sonication for a total of 3 h. For homogeneous and single-layered GO dispersion, the resulting suspension was centrifuged at 16,000 rpm for 30 min. The exfoliated GO was obtained in the supernatant as a brown color. Afterwards, 1.2 g NaOH and 1.0 g chloroacetic acid ($ClCH_2COOH$) were added to 10 mL GO suspension and continually bath-sonicated for another 2 h to efficiently convert OH groups on the oxidized GO to COOH moieties. The GO-COOH suspension obtained was neutralized with diluted HCl, and dialyzed against deionized (DI) water for over 48 h to remove unreacted chemicals and impurities. The lyophilized GO-COOH powder was dispersed in DMSO (1 mg/mL) and consecutively conjugated with 10 mM cystamine dihydrochloride (CTD) using standard EDC/NHS activation chemistry, where the reacting solution was stirred for 2 h and dialyzed against DI

water for over 48 h to remove unbound materials. Finally, 10 mM chlorin e6 (Ce6) was conjugated to amine-terminated GO using the EDC/NHS mediated coupling reaction, and consecutively stirred and dialyzed against DI water (pH 8.5) for over 48 h to obtain the final product, GO-SS-Ce6. To calculate the concentration of Ce6 conjugated on GO, the absorbance of GO-SS-Ce6 (dissolved in 0.1 M NaOH / 0.1% SDS) was measured at 400 nm and the absorbance of GO at the same wavelength was then subtracted. Ce6 is known to have a molar extinction coefficient of $1.5 \times 10^5$ $M^{-1}$ $cm^{-1}$ at 400 nm.[1]

**Characterization of GO-SS-Ce6 Conjugate**

For the analyses of optical properties, GO and GO-SS-Ce6 were dispersed in PBS (6.7 mM, pH 7.4, NaCl 154 mM), whereas free Ce6 was dissolved in 1% (v/v) Tween/PBS. The UV/Vis spectra of GO, GO-SS-Ce6, and free Ce6 in the aqueous solutions were measured with a UV/Vis scanning spectrophotometer (DU730, Beckman Coulter, Brea, CA). Fluorescence spectra of the samples were recorded on a multifunctional microplate (Tecan, Safire2) with an excitation at 400 nm. The morphological features and size of GO-SS-Ce6 were observed using atomic force microscopy (AFM) in tapping mode to obtain height and phase data, by casting an aqueous solution of GO or GO-SS-Ce6 onto a freshly cleaved silica surface and drying at room temperature. The surface charge and hydrodynamic size of GO-SS-Ce6 were characterized using a zeta potential/particle sizer (Malvern Instrument).

**Analysis of Fluorescence and Singlet Oxygen Generation**

To observe fluorescence quenching and recovery, free Ce6 was dissolved in 1% (v/v) Tween/PBS to prevent the self-quenching effect resulting from aggregation. On the other hand, GO and GO-SS-Ce6 were dissolved in PBS. In this study, a concentration equivalent to 1 μM Ce6 was used. A cleavable disulfide linkage of GO-SS-Ce6 permits the release of Ce6 with an increasing dithiothreitol (DTT) concentration that apparently results in the recovery of the fluorescence signal. Fluorescence measurements were performed by measuring the intensity of free Ce6 released from the GO-SS-Ce6 conjugate after the addition of 0–1 mM DTT. The excitation wavelength was 400 nm and the emission wavelength was in the range from 430 to 700 nm. The time-dependent fluorescence response was conducted with a time interval of 1 h after adding 0–1 mM DTT. To evaluate the inhibitory and recovery characteristics with respect to singlet oxygen generation, singlet-oxygen-detecting reagent (Singlet Oxygen Sensor Green, Molecular Probes) was dissolved in 1% (v/v) Tween/PBS (saturated with oxygen gas) containing free Ce6, GO, GO-SS-Ce6 conjugate, or GO-SS-Ce6 treated with 1 mM DTT for 3 h. The final concentration of free Ce6 and GO-SS-Ce6 conjugate was maintained at 1 μM. The final concentration of SOSG reagent in the test solution was maintained at 1 μM. Each solution was irradiated

with a CW laser beam at 670 nm (irradiation dose rate 100 mW/cm$^2$). All experiments were performed in triplicate.

### *In Vitro* Cellular Uptake and Fluorescence Activation Test

Glutathion-rich A549 human lung cancer cells were plated at a density of $5 \times 10^4$ cells/well onto a LabTek II Chambered Coverglass (Nalge Nunc International Corp.) and incubated for 24 h to induce cell attachment. Free Ce6 or GO-SS-Ce6 was added to fresh RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin with an equivalent concentration of 1.5 μM Ce6. After incubation for 24 h, the cells were washed three times and fresh culture medium was added. Fluorescence images with an excitation of 400 nm were acquired using a confocal laser scanning microscope (CLSM, ZEISS LSM 510 META). For labeling lysosomes of cells with LysoTracker Green, A549 cells were prepared with a population of $5 \times 10^4$ cells/well onto a LabTek II Chambered Coverglass. After 24 h incubation for cell attachment, GO-SS-Ce6 dissolved in cell culture medium was added and incubated for another 24 h in a humidified 5% $CO_2$ incubator at 37 °C. Afterwards, 150 nM LysoTracker Green was added to the cells for 30 min and then fluorescence images were acquired using CLSM. To quantify the intracellular uptake of free Ce6 and GO-SS-Ce6, flow cytometric analysis (FACS) equipped with an excitation laser at 633 nm was used. A549 cells were grown with a density of $1.0 \times 10^5$ cells/well onto six-well plates and incubated for 24 h. Then, the existing cell culture medium was replaced with a fresh one containing free Ce6 or GO-SS-Ce6 at the equivalent concentration of 1.5 μM Ce6. After the cells had been incubated for 24 h, they were washed three times with cell culture medium, harvested, and transferred to FACS tubes.

### *In Vitro* Phototoxicity Test

A549 cells were seeded in a 96-well plate at $1 \times 10^4$ cells/well and incubated for 24 h. Then, free Ce6 and GO-SS-Ce6 were diluted with a cell culture medium containing 10% FBS to obtain equivalent concentrations of 0.25 μM, 0.5 μM, 1.0 μM, and 1.5 μM Ce6. The cells were subsequently treated with the samples for 24 h. For the untreated control group, the same volume of fresh culture medium without photosensitizer was added to the plate. After the cells had been washed three times, a fresh culture medium was added. The PDT-treated groups were irradiated with a 670 nm CW laser beam at a dose of 20 J/cm$^2$ and at a dose rate of 150 mW/cm$^2$. The cells were incubated for an additional 24 h and cell viability was measured using a cell counting kit-8 (Dojindo Laboratories). Cell viability was calculated as a percentage relative to untreated control cells. Dark toxicity of the free Ce6 and GO-SS-Ce6 was also evaluated by incubating these compounds for 24 h at the same concentration without light treatment.

**Statistical Analysis**

Unless otherwise specified, statistical analysis was done using a Student's t-test. Differences were considered statistically significant when the P-value was less than 0.05. Results are expressed as mean ± SD.

**References**

[1] M. R. Hamblin, J. L. Miller, I. Rizvi, *Cancer Res.* **2001**, *61*, 7155-7162.