Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2011

Supporting Information

Biocompatible reduced graphene oxide prepared by using dextran as a multifunctional reducing agent

Young-Kwan Kim, Mi-Hee Kim and Dal-Hee Min*

Department of Chemistry, Institute for the BioCentury, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Korea

Materials and Methods

Materials

Dextrans (from leuconostoc ssp., Mr ~ 6000, ~ 15000-25000 and ~ 40000) and Cetyltrimethylammonium bromide (CTAB) were purchased from Fluka Chemical Corp. (Milwaukee, WI, USA). Natural graphite (FP 99.95% pure) was purchased from Graphit Kropfmühl AG (Hauzenberg, Germany). Sodium nitrate and hydrogen peroxide (30% in water) were purchased from Junsei (Japan). Potassium permanganate, ammonium hydroxide (NH_3, 28~30% in H_2O) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), 3-aminopropyltriethoxysilane (APTES), sodium dodecyl sulfate (SDS), Triton x-100 and anhydrous dimethylformamide (DMF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). CCK-8 (cell counting kit-8) was purchased from Dojindo (Rockville, USA). Nitric acid and sulfuric acid were purchased from Samchun (Seoul, Korea). 10x PBS (Phosphate-Buffered Salline. 10x), DMEM (Dulbecco's Modified Eagle's Medium) and FBS (fetal bovine serum) were purchased from WelGENE Inc. (Seoul, Korea). Ethanol was purchased from Merck (Darmstadt, Germany). 500-nm SiO_2/P^{++} Si substrates (500 μm in thickness) and were purchased from STC (Japan). All chemicals were used as received.

Preparation of the graphite oxide

Natural graphite (3 g) and $NaNO_3$ (1 g) were dissolved in 46 mL of H_2SO_4 with stirring in an ice bath. Next, 6 g of $KMnO_4$ was gradually added into the mixture with stirring while maintaining the temperature below 20°C. The temperature of the reaction mixture was then raised to 35°C and the mixture was suspended for an hour with stirring. Distilled water (80 mL) was added to the mixture, stirred for 30 min and diluted with an additional 200 mL of water while maintaining the temperature below 95°C. Finally, 6 mL of H_2O_2 (30%) was added drop-wise. The mixture was filtered and washed with copious amounts of water until the filtrate was neutral. The filter cake was dried under vacuum for 48 h at room temperature.

Synthesis of dextran-reduced graphene oxide (D-RGO)

50 mg of graphite oxide was dispersed in 50 mL of 0.1 % aqueous dextran solution by sonication for 30 minutes. 25 μL of ammonium hydroxide was added to the graphene oxide (GO) suspension and the mixture was heated at 95 ^{o}C for 3 hours with stirring. After reduction, the D-RGO was purified and collected by repeated centrifugation and re-suspension in water.

Synthesis of D-RGO/gold nanocomposite

28 μL of 250 mM $HAuCl_4$ was added to boiling D-RGO suspension (10 mL at 0.1 mg/ml) at 95 ^oC and stirred for 15 min. After reaction, the reaction mixture was purified by repeated centrifugation and washing with water to collect D-RGO/gold nanocomposite.

Preparation of GO and RGO papers

20 mL of GO and D-RGO suspension (1 mg/mL) was diluted with 300 mL of water and filtered through mixed cellulose ester membrane (450 nm). The membrane with filtered GO and D-RGO was dried at 60 ^oC for 6 hours and dissolved in acetone for 30 min to make the filtered GO and D-RGO papers free from membrane. The D-RGO paper was annealed at 500 ^oC for 2 hours under argon atmosphere for removal of dextran between each D-RGO sheet.

Preparation of MTT stock solution

MTT powder was dissolved in 1x PBS at 5mg/mL concentration and the solution was filtered to sterilize through a 0.2 μm syringe filter. The stock solution was stored in -20 ^oC, covered with aluminum foil.

Cell Culture

HeLa (human cervical cancer) cells were cultured and harnessed for cytotoxicity assay. Growth media was Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5g/L D-glucose and supplemented with 10% FBS (fetal bovine serum), 100 units/mL penicillin, and 100 ug/mL streptomycin. The cells were grown in a humidified 5% CO_2 incubator at 37 ^oC.

MTT assay for Viability test

HeLa cells were seeded at 10,000 cells per well of a 96-well culture plate with 100 μL of growth media (about 50-70 % confluency for the experiments). After GO and RGO at concentrations ranging from 0~450 μg/ml in PBS were added and incubated for 24 hours at 37°C. Following GO and RGO treatment, cells were rinsed with 1X PBS and treated with 20μL MTT reagent in serum-free media. The cells were incubated for 2 hours until purple color develops indicating that MTT was to be metabolized. The media were discarded, and 200 μL DMSO was added to each well to make insoluble formazan salt solublized. Then the optical densities of each well in the plates were read at 570 nm. Mean and standard deviation for the triplicate wells were recorded by UV-vis spectrophotometer.

CCK-8 assay for Viability test

CCK-8 assay uses WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-etrazolium, monosodium salt], and produces water-soluble formazan dye when WST-8 is reduced by dehydrogenases in cells. HeLa cells were seeded at 10,000 cells per well of a 96-well culture plate with 100 μL of growth media (about 50-70 % confluency for the experiments). After GO and RGO at concentrations ranging from 0~450 μg/ml in PBS were added and incubated for 24 hours at 37°C. Following GO and RGO treatment, cells were rinsed with 1X PBS and treated with 10 μL CCK-8 reagent in serum-free media. The

This journal is (c) The Royal Society of Chemistry 2011

cells were incubated for an hour until orange color develops indicating that WST-8 was to be metabolized. Then the optical densities of each well in the plates were read at 450 nm. Mean and standard deviation for the triplicate wells were recorded by UV-vis spectrophotometer.

LIVE/DEAD cell stain for Biocompatibility Test

The biocompatibility of GO and RGO films as substrates were tested by examining the growth of HeLa cells using the LIVE/DEAD Viability/Cytotoxicity Assay Kit (from Molecular Probes Invitrogen). HeLa cells were seeded on films at 40,000 cells per well of a 24-well cell culture plate (about 50-70 % confluency for the experiments). Following incubation for 40 hours, cells were rinsed with 1X PBS and 200 μL of the combined live-dead cell staining solution (2 μM calcein AM and 4 μM EthD-1 in D-PBS) was added to each well and incubated with cells for 20~30 min. Images were obtained using a BX51M optical microscope (Olympus Co., Japan) equipped with fluorescence light source and filters.

Characterization of the prepared GO, D-RGO and D-RGO/gold nanocomposite

The UV-vis spectra were obtained with a UV-2550 (Shimadzu, Japan). Atomic force microscopy (AFM) analysis was performed with an XE-100 (Park system, Korea) with a backside gold-coated silicon SPM probe (M to N, Korea). The large-area observation of D-RGO/gold nanocomposite was carried out by field emission scanning electron microscopy (FE-SEM) (S-4800, Hitachi, Japan). Fourier transform infrared (FTIR) absorption measurements of the graphite and GO were recorded with an EQUINOX55 (Bruker, Germany) by KBr pellet method. Raman spectra of GO, D-RGO and D-RGO/gold nanocomposite were recorded on a LabRAM HR UV/vis/NIR (HORIBA Jobin Yvon, France) using a CW Ar-ion laser (514.5 nm) as an excitation source focused through a confocal microscope (BXFM, Olympus, Japan) equipped with an objective lens (50 x, numerical aperture = 0.50). High-resolution X-ray photoelectron spectroscopy (XPS) was performed on an ESCA 2000 (Thermo VG Science, USA) with a twin X-ray source (Mg/Al target). The sheet resistances of the GO, D-RGO and annealed D-RGO papers were obtained by a standard 4 probe method and were measured with a CMT-SR2000 (Changnam Tech, Korea). Images of HeLa cells treated with GO, D-RGO and RGO derivatives were collected using a Ti inverted fluorescence microscope equipped with a 60 x (numerical aperture = 1.4) objective (Nikon Co., Japan), a CoolSNAPcf charge-coupled device (CCD) camera (Photometrics, Tucson, AZ) using Metamorph image analysis software (Molecular Devices, Sunnyvale, CA, USA). Images of HeLa cells on GO and D-RGO papers were captured with a BX51M optical microscope (Olympus Co., Japan) equipped with fluorescence light source and filters.

Full author list of reference (5) Hernandez, Y. et. al. Nat Nanotechnol. 2008, 3, 563-568.

Hernandez, Y.; Nicolosi, V.; Lotya, M.; Blighe, F. M.; Sun, Z.; De, S.; McGovern, I. T.; Holland, B.; Byrne, M.; Gun'Ko, Y. K.; Boland, J. J.; Niraj, P.; Duesberg, G.; Krishnamurthy, S.; Goodhue, R.; Hutchison, J.; Scardaci, V.; Ferrari, A. C.; Coleman, J.N.

Data

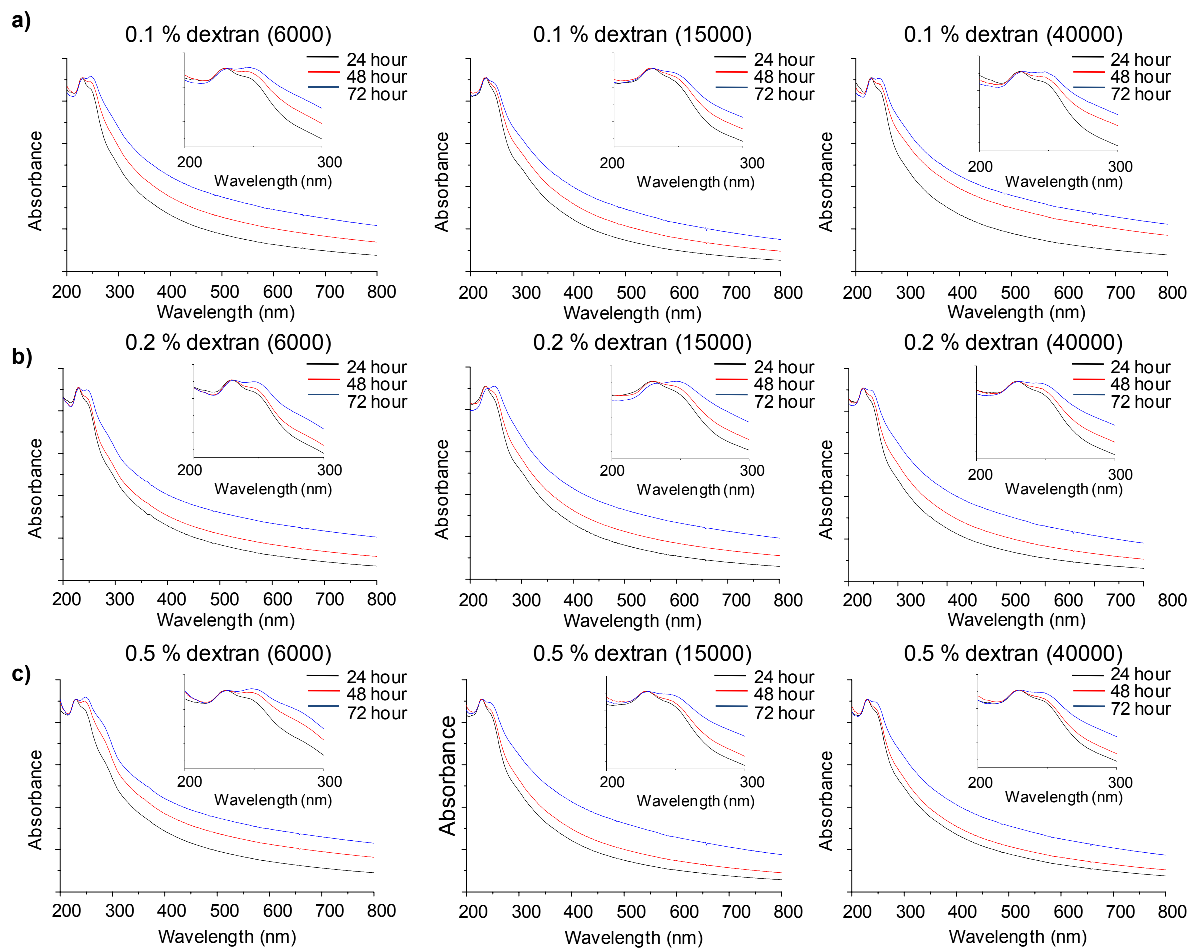

Figure S1. The changes of UV-vis spectra of GO induced by reflux in dextran solution with varying concentrations from 0.1 (a), 0.2 (b) and 0.5 % (c) for 74 hours.

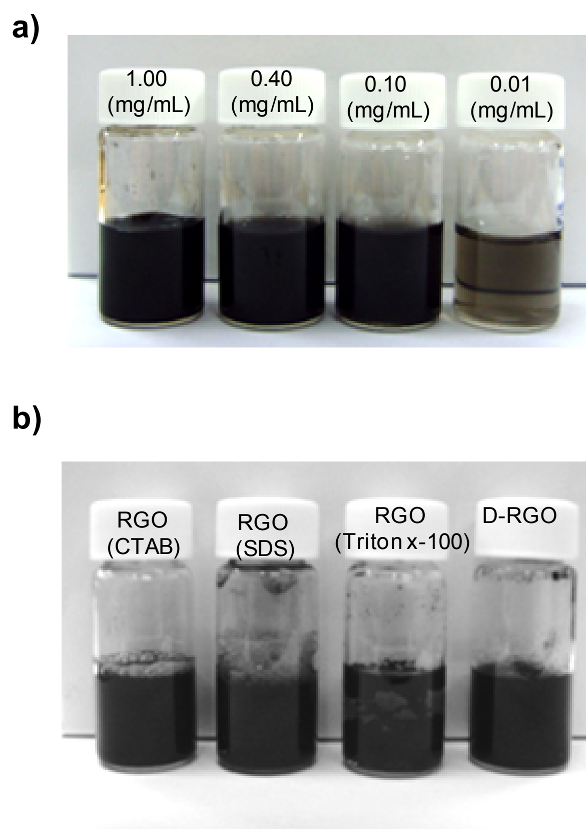

Figure S2. Photographs of D-RGO dispersed in water with varying concentrations from 0.01 to 1.00 mg/mL (a) and D-RGO and RGO derivatives functionalized with CTAB, SDS and Triton x-100 and dispersed in PBS buffer at 1.00 mg/mL concentration (b). All the D-RGO and RGO derivatives were stable in PBS buffer except RGO functionalized with Triton x-100.

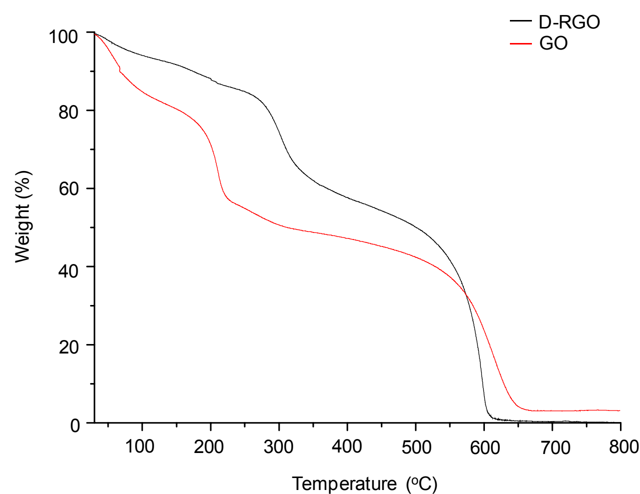

Figure S3. TGA curves of GO and D-RGO. The weight loss of GO around 100°C and 225°C was 15.3 % and 42.6 % from evaporation of adsorbed water and gasification of labile oxygen-containing functional groups on GO. After dextran reduction and functionalization, the weight loss of GO around 100°C and 225°C was decreased to 6.0 % and 13.9 % because of the decreased hydrophilicity and oxygen-containing functional groups induced by dextran reduction. The weight loss of D-RGO around 280°C might be attributed to thermal degradation of dextran.[1] The significant weight loss of GO and D-RGO around 600°C was derived from the thermal degradation of carbon skeleton.[2]

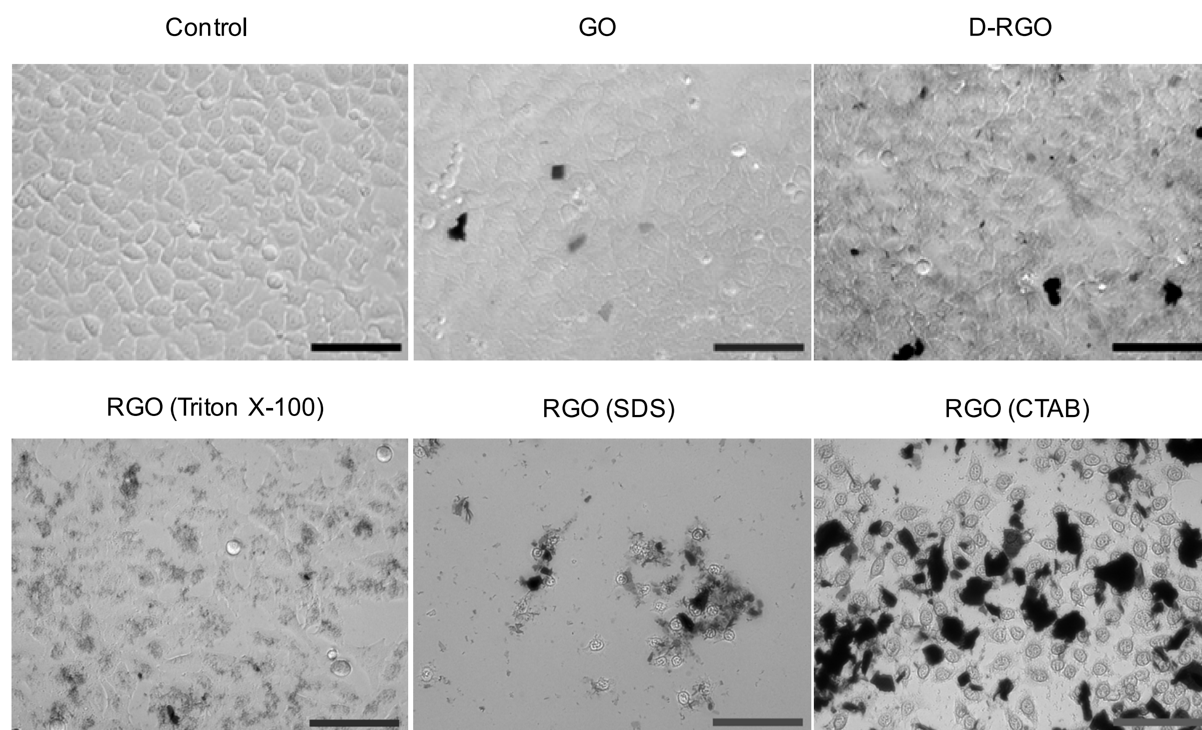

Figure S4. Optical images of HeLa cells exposed to GO, D-RGO and RGO derivatives for 24 hours at 112.5 μg/mL concentration. The morphology and confluency of HeLa cell was not significantly changed by exposure to GO and D-RGO but the HeLa cells exposed to RGO derivatives functionalized with surfactants showed considerable shrinkage and decreased confluency. The black spots on the cell images were attributed to aggregation and insertion of D-RGO and RGO derivativs around HeLa cells. This result also confirmed the high biocompatibility of D-RGO.

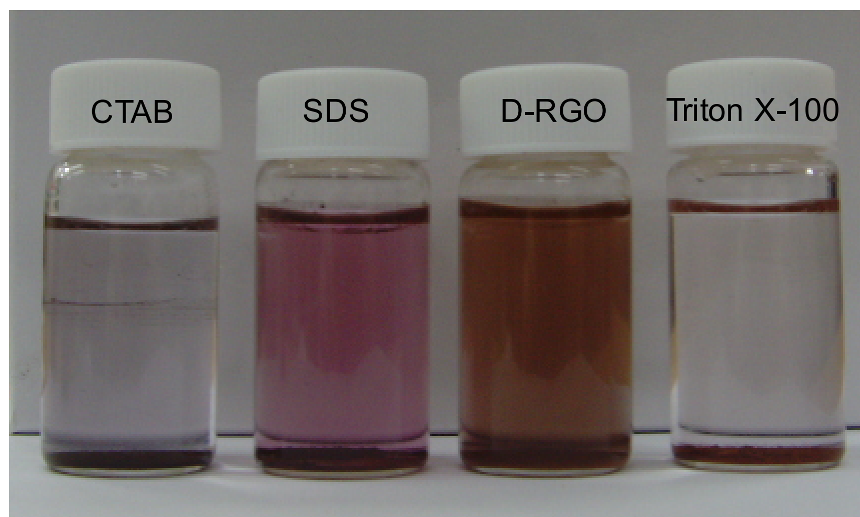

Figure S5. Photograph of suspended D-RGO and RGO derivatives functionalized with various surfactants after reflux with $HAuCl_4$ solution at elevated temperature. The CTAB, SDS and Triton x-100 functionalized RGO suspensions made precipitates at the bottom of glass vials after mixing with $HAuCl_4$ solution but the supernatant of SDS functionalized RGO suspension showed red color by formation of small gold nanoparticles induced by excess SDS. In contrast, there was no precipitate on the bottom of vial containing D-RGO suspension and the suspension of D-RGO/gold nanocomposite was stable for several weeks. This result also confirmed that D-RGO is compatible to aqueous process for nanocomposite synthesis.

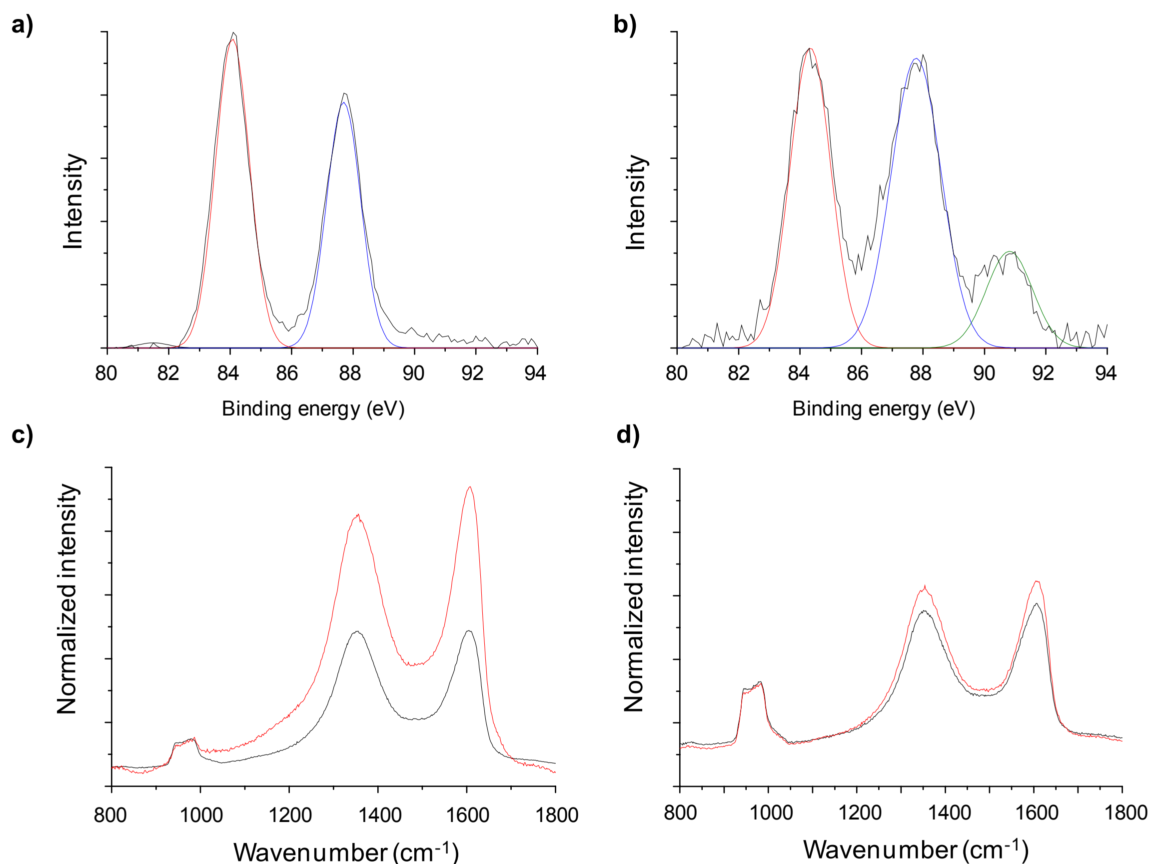

Figure S6. XPS spectra in the Au(4f) region of D-RGO (a) and GO (b) reacted with $HAuCl_4$ at elevated temperature as surface-immobilized thin films on APTES treated SiO_2/Si substrates. There were broad peaks around 84.3 and 90.8 eV corresponding to small cluster of Au and Au^{3+}, respectively, which indicated incomplete reduction of gold salt (b). In contrast, XPS spectrum of D-RGO/gold nanocomposite showed relatively sharp peaks at 84.0 and 87.7 eV and no peak from Au^{3+} (a).[3] This result implied that D-RGO was successfully acted as a template for gold nanoparticle formation. In addition, the Raman signal of D-RGO was significantly increased by formation of gold nanoparticles on its surface compared to GO sheets refluxed with gold salt (c, d).

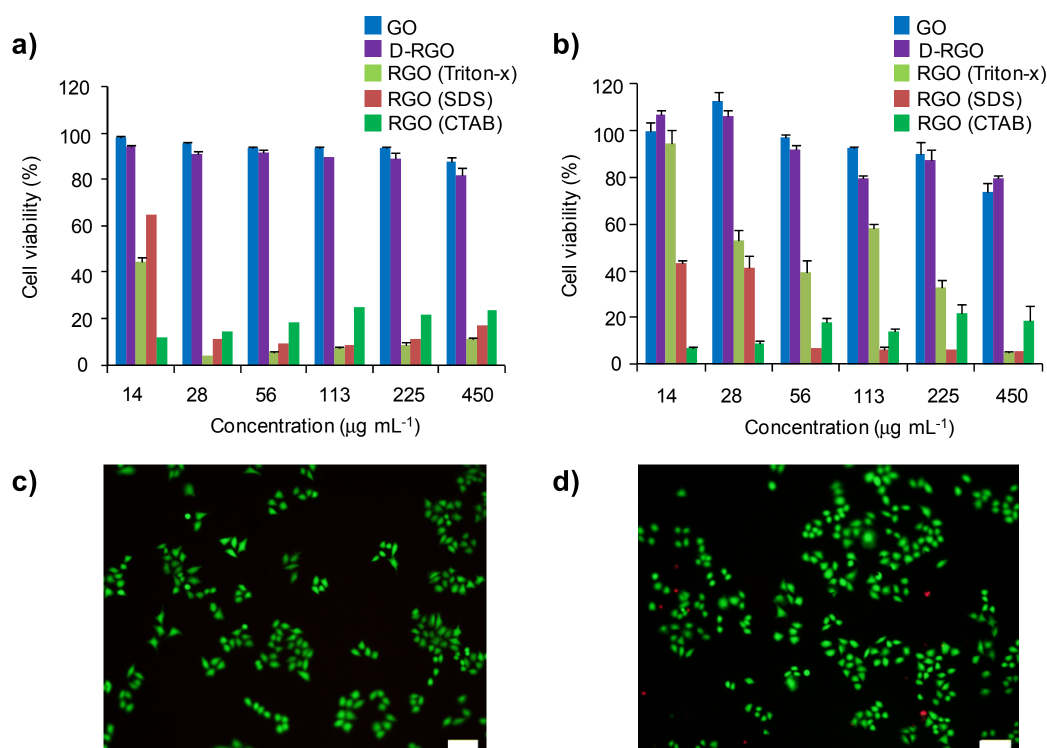

Figure S7. Cell viability of HeLa cells exposed to GO and RGO derivatives obtained by (a) MTT and (b) CCK-8 assays. Fluorescence micrographs of HeLa cells stained by a live/dead assay kit on (c) GO and (d) D-RGO paper. Green and red fluorescence indicate live and dead cells, respectively. The scale bars are 100 μm.

Reference

(1) Khan, F.; Mann, S. *J. Phys. Chem. C* **2009**, *113*, 19871
(2) Zhu, C.; Guo, S.; Fang, Y; Dong, S. *ACS Nano* **2010**, *4*, 2429
(3) Miyama, T.; Yonezawa, Y. *Langmuir*, **2004**, *20*, 5918-5923.