Supplementary materials

Near infra-red photoluminescent graphene nanoparticles greatly expands use in noninvasive biomedical imaging

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Experimental:

Methods

Materials. Pitch carbon fiber was purchased from Fibre Glast Development Corporation (Carr Drive Brookville, OH). Sulfuric acid, Nitric acid, Sodium hydroxide, sodium carbonate was purchased from Sigma-Aldrich (St. Louis, MO). Cell culture reagents, including fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), penicillinestreptomycin, trypsin/EDTA, and Dulbecco's phosphate buffer saline (PBS) were purchased from Gibco BRL (Carlsbad, CA, USA). 3-(4,5-dimethylthiazol-2-yl)-2 and 5-diphenyl tetrazolium bromide (MTT) were obtained from Amresco Inc. (Solon, OH, USA).

Synthesis of Photoluminescent Graphene Nanoparticles. The synthesis process is represented in the schematic diagram in figure S1. The amount of carbon fiber (CF), sulfuric acid, nitric acid and water with other reaction parameter is presented in Table S1. In brief, half portion of sulfuric acid and the entire nitric acid were taken in a 3 neck round bottom flask. The three neck flask was placed on a heating mantle with continuous flow of N_2 gas. On the other beaker CF was dissolved in a mixture of remaining sulfuric acid and DI water. The CF containing solution was sonicated by ultrasonicator (Ningbo Haishu Sklon Electronic Instrument Co., Ltd., sonicator83062) for 30 min. Temperature was controlled from 65 to 110 °C depending on the desired photoluminescent graphene (Tablse S1). The solution was sonicated for further 60 min by bath sonicator at room temperature for homogeneous dispersion. The sonicated CF containing solution was injected to the three neck flask and stirred by mechanical stirrer for 12 hr. During stirring temperature was maintained by heating mantle. After completion of reaction required amount of water was added. Sodium hydroxide and sodium carbonate was added as specified amount to make the acidic solution neutralization. Reaction solution was slowly stirred at 0-4 °C for a certain period to remove the precipitated salts. The graphene nanoprticles containing solution was collected by decantation and freeze dried for 48 hr.

Characterization of photoluminescent graphene nanoparticles. The size distribution and morphologies of near infrared graphene nanoprticles were characterized using dynamic light scattering (DLS) (ELS-Z2, Otsuka Electronics Co., Ltd, Japan) and a SEM (JEOL, Japan), respectively. The TEM image and the selected area electron diffraction pattern were obtained on a JEOL 2100 Field Emission Gun TEM. Photo-luminescent, excitation and emission were measure by luminescent analyzer FluoroMate FS-2 (Scinco, Korea). The XRD data were collected on a Rigaku D/Max Ultima II Powder X-ray diffractometer. XPS analyses were carried

out on a PHI Quantera x-ray photoelectron spectrometer with a chamber pressure of 5×10-9 torr and an Al cathode as the X-ray source. The source power was set at 100 W, and pass energies of 140.00 eV for survey scans and 26.00 eV for high-resolution scans were used. Band gap values of near infrared graphene nanoprticles was measured based on absorbance and reflectance the respective graphene nanoprticles. The calculation methods has been elaborately discussed elsewhere.

Cytotoxicity evaluation. *In vitro* cytotoxicty study of near infrared graphene nanoprticles has been examined in MDA-MB231 cell for 24 and 48 hr. At 37 ^{o}C and 5 % CO_2 containing humidified atmosphere, cells were grown in a medium containing MEM with 10 % fetal calf serum. The cells (5×104 cells/mL) grown as a monolayer were harvested by 0.25 % trypsin-0.03 % EDTA solution. 200 µL of cells containing medium were placed in 96 well plates and incubated for 24 hr. After 24 hr, the complete medium was suctioned and sample was added in to the well at different concentrations (10, 25, 50, 100 and 200 µg/ml) with complete medium. MTT solution aliquots at 5 mg/mL in PBS were prepared followed by culture incubation with this solution at 5 % in the culture medium for 4 hr in an incubator with a moist atmosphere of 5 % CO_2 and 95 % air at 37 ^{o}C. After 4 hr, 100 µL of MTT solubilizing solution was added and made a gentle shake for 15 min. Finally, the absorbance of MTT colorimetric assay was measured by Varioskan flash (Thermo Scientific, USA) at a wavelength of 570 nm. The viable quantity of cells was calculated by the following equation:

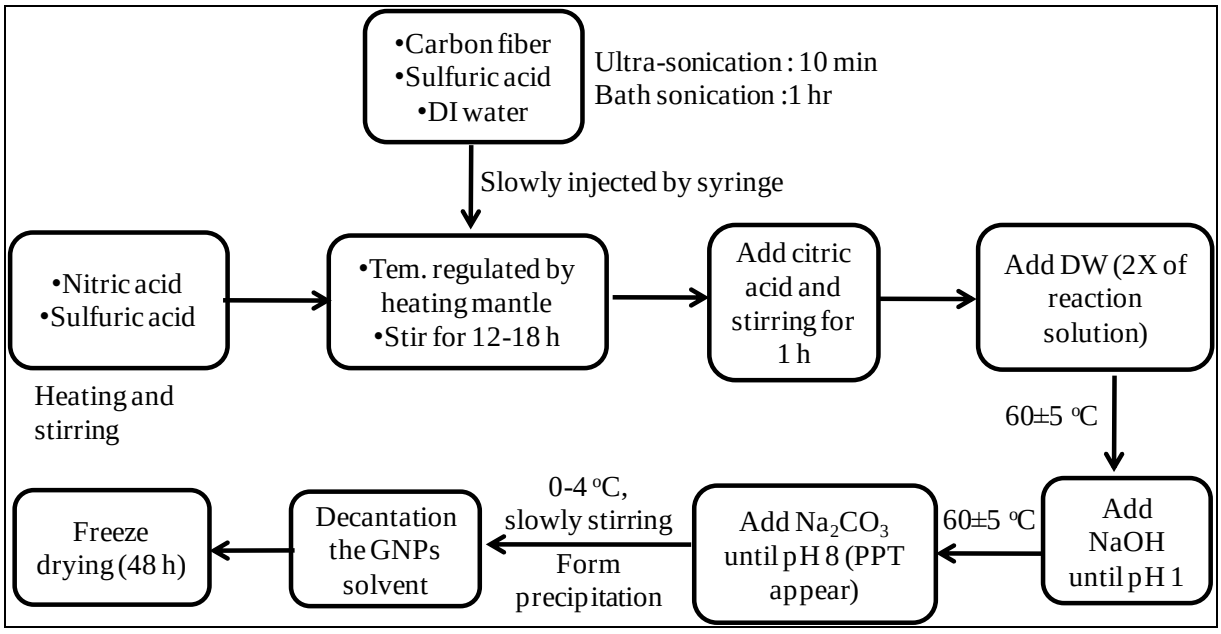
Cell viability (%) = (absorbance of sample cells/absorbance of control cells)×100

***In vitro* cellular uptake.** For a cellular uptake study of near-IR GNPs, the nanoparticles were incubated with MDA-MB231 cell line. The MDA-MB231 cells were cultured at 37 ^{o}C in a

humidified atmosphere containing 5 % CO_2 in a MEM medium with 10 % fetal bovine serum. The cells (5×10^4 cells/mL) grown as a monolayer were harvested by 0.25 % trypsin-0.03 % EDTA solution. The cells (200 μL) in their respective media were seeded in an 8-well plate and pre-incubated for 24 hr before the assay. The GNPs (0.1 mg/mL) was added with the 8-well plate and incubated for 1 hr before observation by a confocal laser scanning microscope (CLMS). The wells were washed 5 times by PBS to remove the free particles from the outside of the cell membrane. A 4% formaldehyde solution was added to preserve the cell, and observed by CLMS to get a noise free clear cellular image.

Animal experiments. Six to seven-week-old SKH1 female nude mice (average body weight of 21-25 g) were purchased from Orient Bio INC., (Seoul, Korea) and maintained under specific pathogen-free conditions. All experiments were approved by institutional guidelines of the Institutional Animal Care and Use Committee (IACUC) of the Catholic University of Korea College of Medicine in accordance with the NIH Guidelines. For in-vivo imaging studies, SKH1 mice were administered 2.5 mg/kg of near infrared graphene naoparticles through tail vain. Mice were anesthetized with ketamine (87 mg/kg, Virbac Laboratories, France) and xylazine (13 mg/kg, Kepro B.V., Netherland) via intraperitoneal injection. In vivo mice images were taken by a time-domain diffuse optical tomography system. In experimental section, mice were placed on the imaging platform. Images were taken at 0.5, 1, 2, 4, 8, and 16 hr of post injection. The 3D scanning region of interest was selected using bottom-view CCD. All images were taken by using the Kodak *in vivo* imaging system (4000MN PRO, Kodak, USA). The ex-vivo images of organs were taken after dissection of the mice. The organs were isolated after 16 hr observation.

Supporting figures:



Scheme S1. The schematic diagram represents the synthesis process of photoluminoscent graphene nanoparticles.

Table S1. Controlling of reaction parameters to synthesis of different graphene nanoparticles based on excitation.

Carbon fiber (mg)	$H2SO_4$ (99%)(mL)	HNO_3 (60%)(mL)	DI water (ml)	Citric acid (mg)	Rxn. Temp. (C)	Excitation (nm)
100	70	20	X	100	105±5	450
100	80	20	X	100	95±5	550
100	90	25	5	100	85±5	655
100	100	25	10	100	75±5	705
100	100	30	10	100	65±5	805

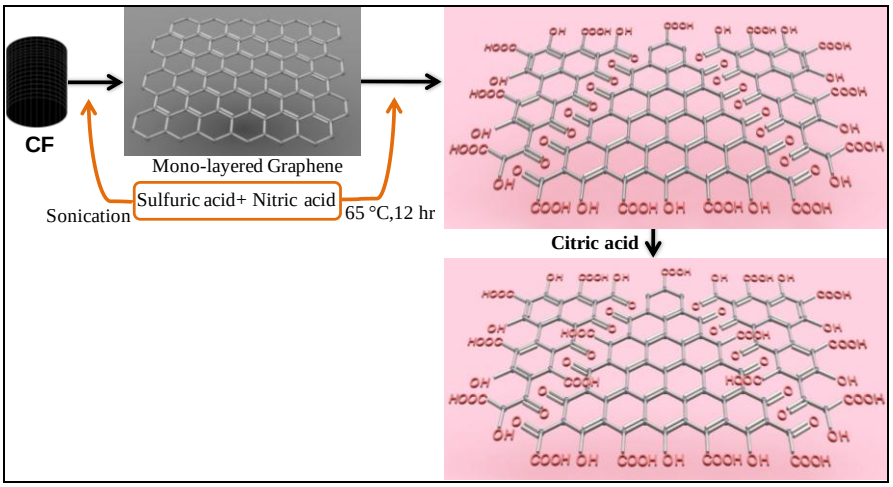


Figure S1. Synthesis process of highly photoluminoscent graphene nanoparticles from carbon fiber.

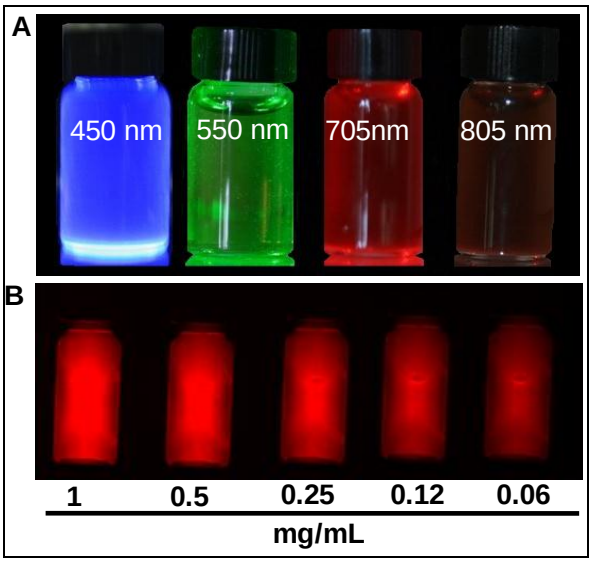


Figure S2. Optical properties of graphene nanoparticles. The as synthesized graphene nanoparticles shows different excitation properties with different colors (a) and optical emission of near infrared graphene nanoprticles at different concentrations (b).

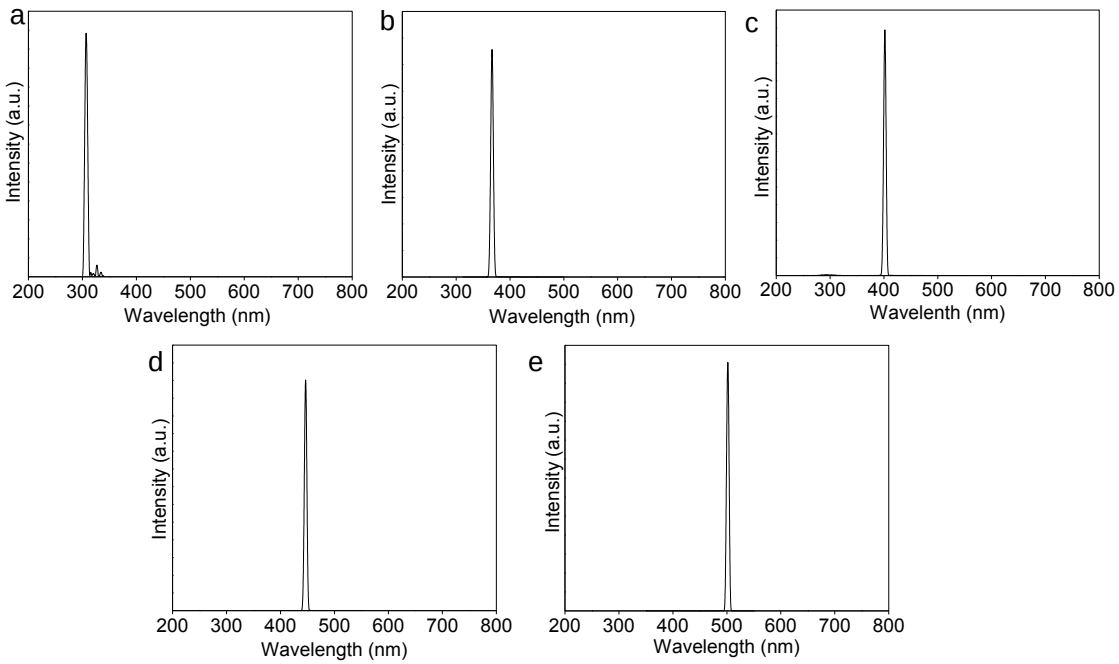


Figure S3. Fluorescence excitation spectra of GQDs with emission at (a) 455 nm, (b) 555 nm, (c) 650 nm, (d) 705 nm and (e) 805 nm.

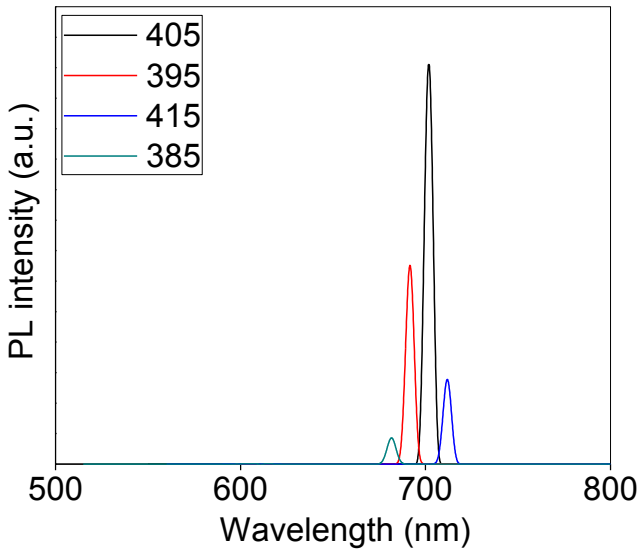


Figure S4. Fluorescence spectra of near-IR GQDs, registered at different excitation wavelength.

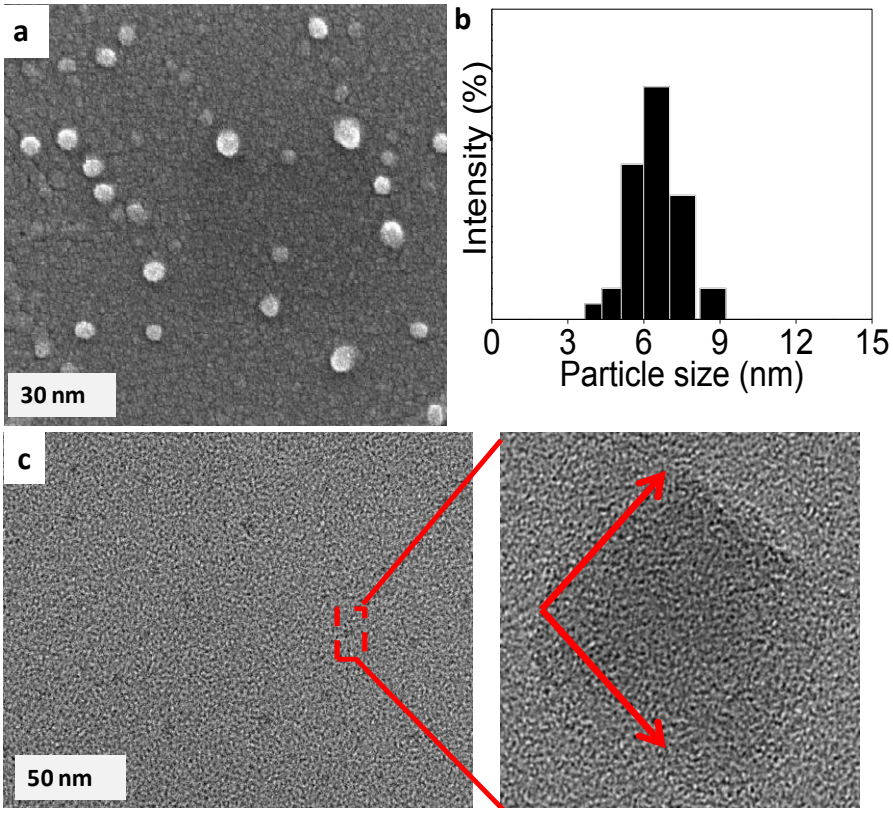


Figure S5. Size and morphologies of near infrared graphene nanoparticles. (a) Field emission scanning electron microscopy, (b) size distribution from dynamic light scattering, (c) transmission electron microscopy image of graphene nanoparticles (emission 705 nm). The magnified image confirms the zig-zag shape of graphene nanoparticles.

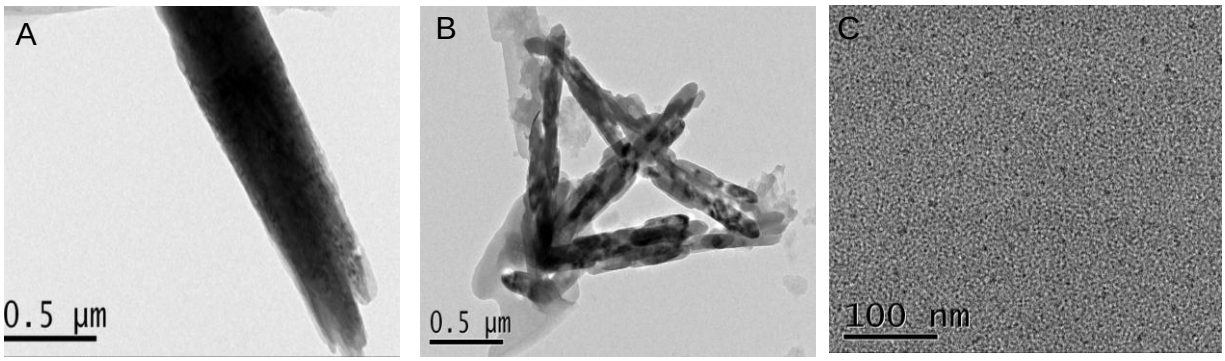


Figure S6. Morphology of CF and its' derivatives. TEM images of carbon fiber (a), after sonication of CF for 10 min (b) , and nano-sized graphene particles (c). The carbon fiber shows rod type with around 0.5μm in width.

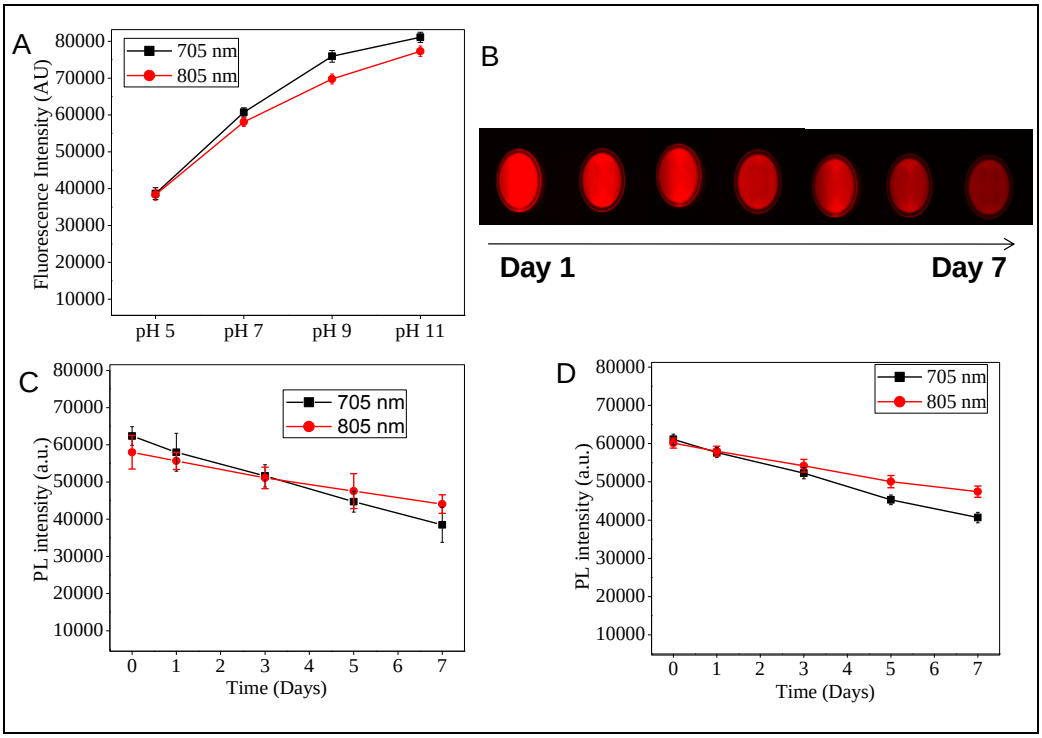


Figure S7. PL stability. Stability of near infrared graphene nanoparticles was investigated in different conditions; pH buffer (a), optical stability measured by kodak molecula imaging station (b) in PBS (c), and 10% FBS solution (d).

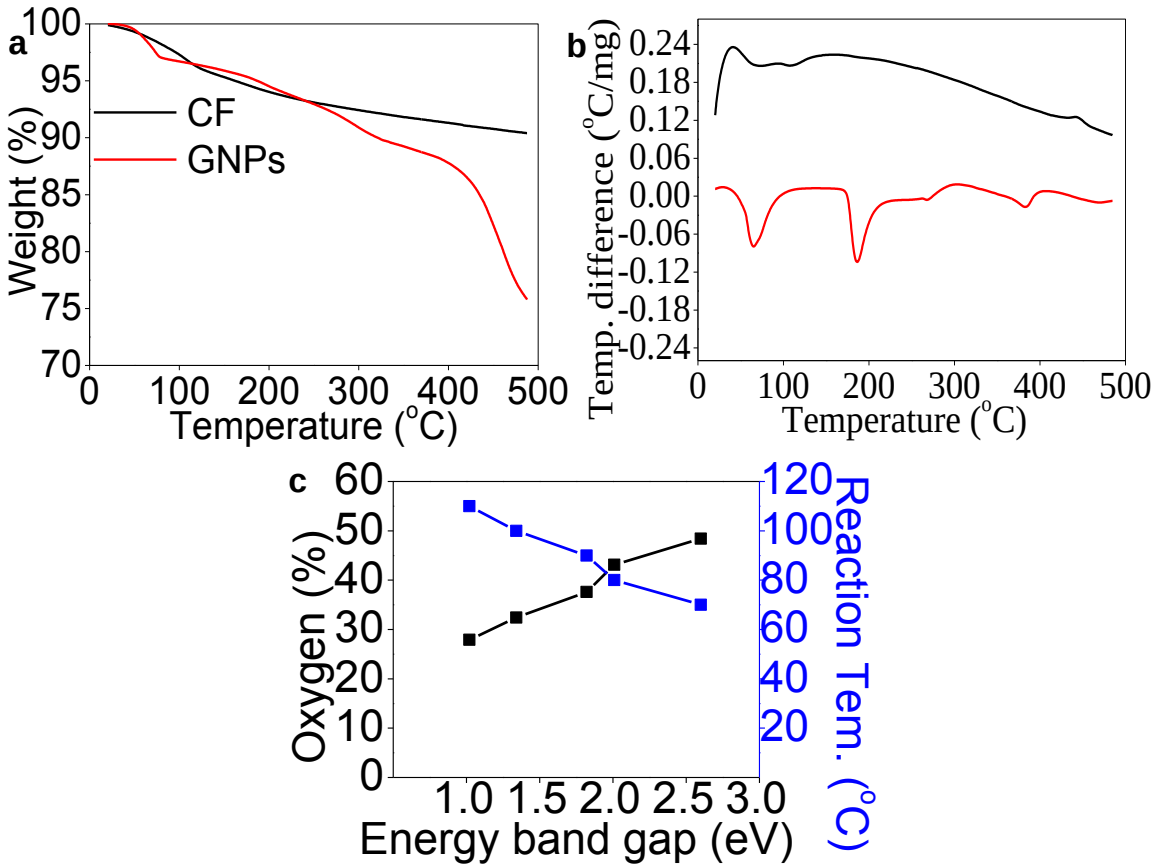


Figure S8. Differential thermal analysis. (a) Characterization of thermal properties measured by TGA shows variation between CF and NIR graphene nanoparticles. Thermal properties of CF and near infrared graphene nanoprticles measured by DTA (b). (c) Variation of energy bad gap of the as synthesized nanoparticles regarding reaction temperature and presence of oxygen atom with graphene.

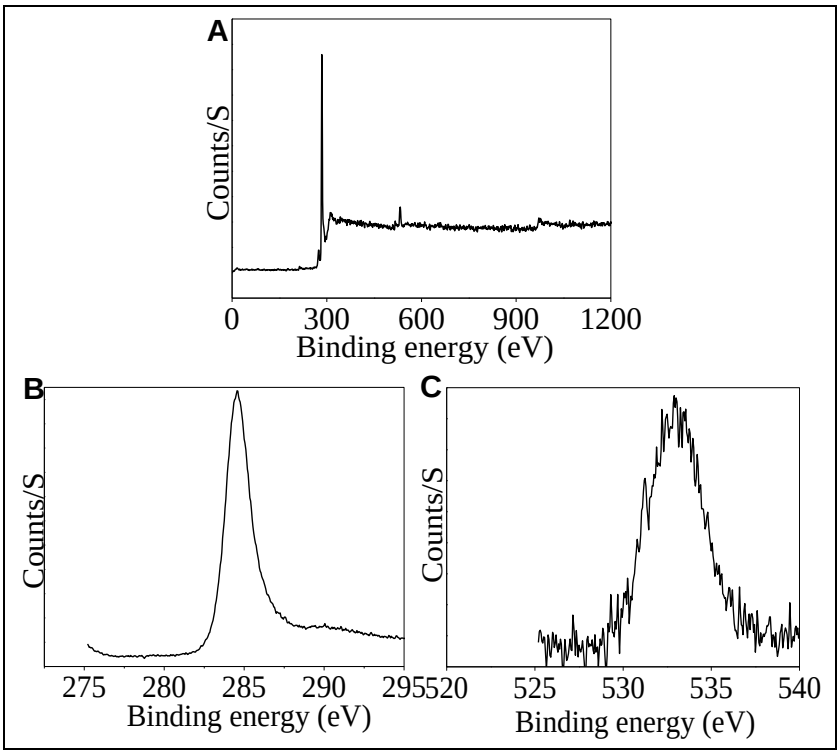


Figure S9. XPS survey scan of CF. (a) XPS scanning spectrum shows two major peaks of carbon and oxygen. High resolution XPS survey scan show (b) C1s (C=95.96%), and (c) O1s (O=4.04%). The spectrum demonstrate that the starting martials carbon fiber is composed carbon with partial amount of oxygen. The carbon fiber does not shows any band gap, exciation and emission properties.

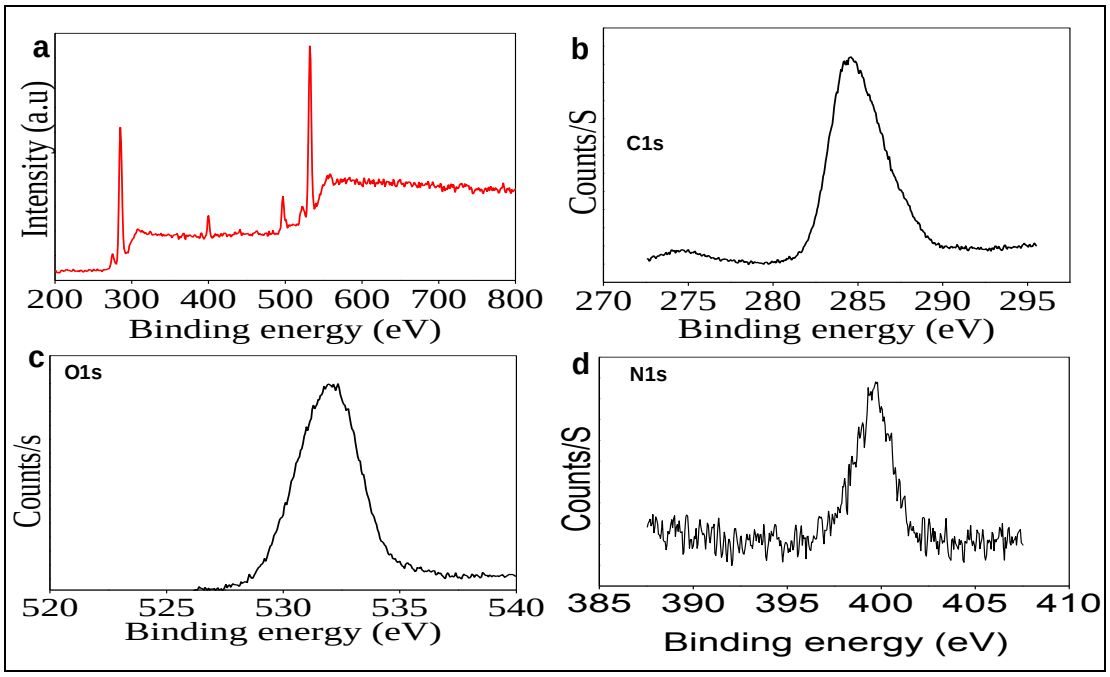


Figure S10. XPS survey scan of near infrared graphene nanoparticles. (a) XPS scanning spectrum shows two major peaks of carbon and oxygen. High resolution XPS survey scan show (b) C1s (C=56.04%), (c) O1s (O=43.16%) and (d) N1s (0.80 %). The composition of GNPs are different than that of CF as observed from the survey. The as synthesized GNPs contains around 43% oxygen which is responsible for band gap, excitation and emission properties.

Table S2. XPS scanning survey of carbon fiber (CF) and photolumunoscence graphene nanopartiles (GNPs) and their energy band gaps.

Element	CF	GNPs-450	GNPs-550	GNPs-655	GNPs-705	GNPs-805
Carbon	95.9	71.5	67.3	62.1	56.04	51.2
Oxygen	4.04	27.9	32.4	37.6	43.1	48.4
Nitrogen	X	0.51	0.21	0.23	0.8	0.3
Band gap (eV)	X	1.02	1.34	1.82	2.01	2.63

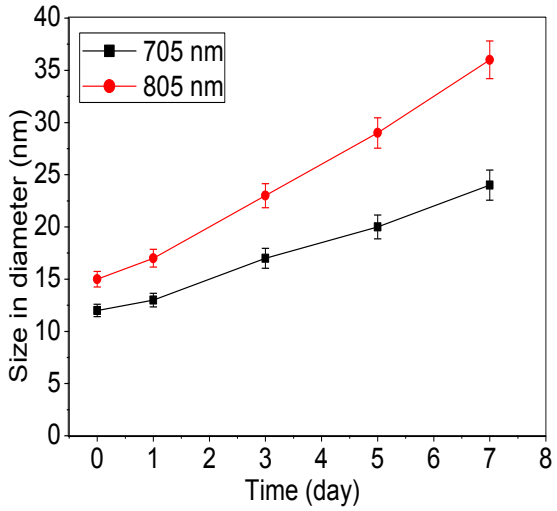


Figure S11. Size stabilitty. Size stability of near infrared graphene nanoparticles in 10% FBS for 7 days shows no mentionable changes in size measured by DLS.

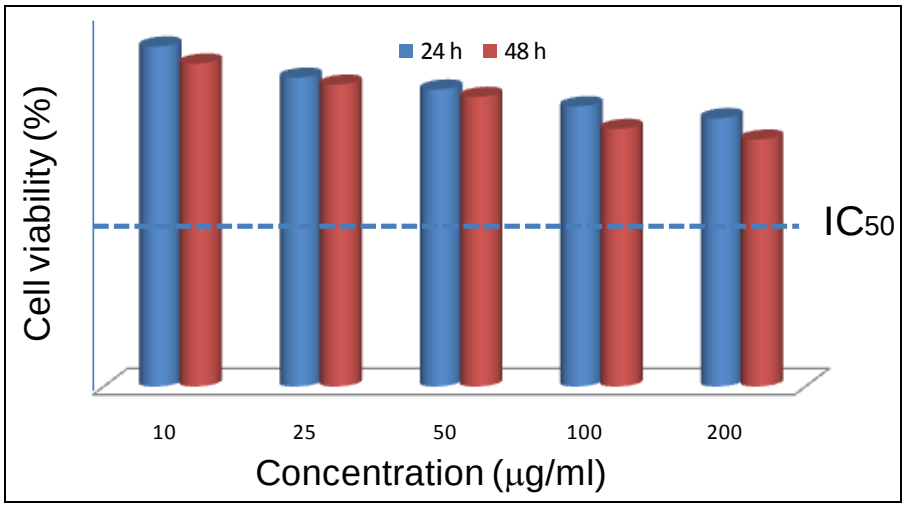


Figure S12. *In vitro* cytotoxicity. *In vitro* cytotoxicity assay of near infrared graphene nanoparticles in MDA-MB231 cancer cell line for 24 hr and 48 hr represents no mentionable toxicity.

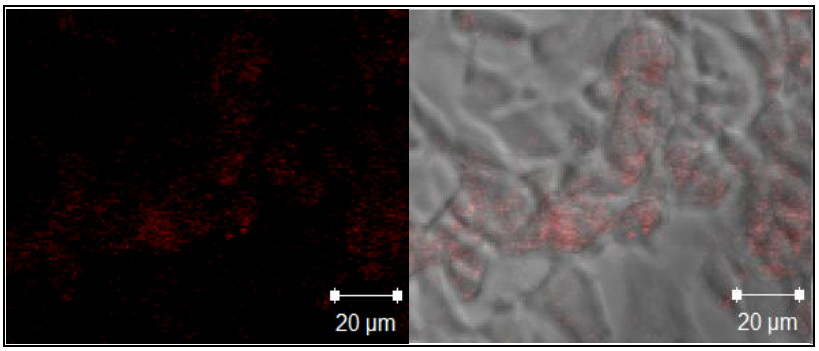


Figure S13. Cellular uptake of GNPs. MDA-MB 231 cancer cells were incubated with 0.1mg/mL of GNPs at 37°C for 2 hr. The cell images were analyzed for determining cell permeability of GNPs using confocal laser microscopy.

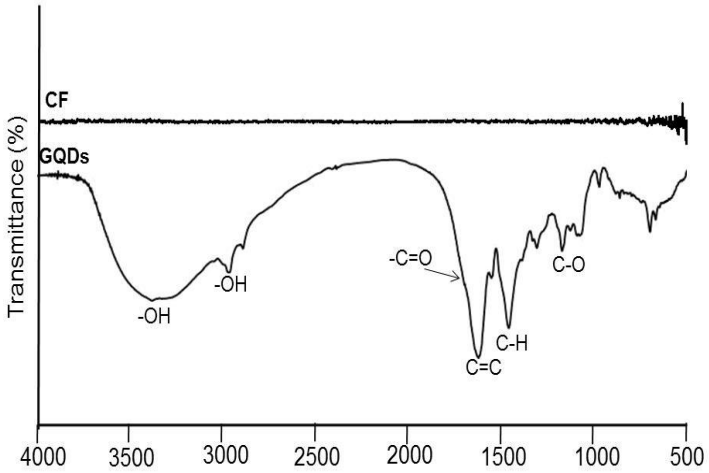


Figure S14. FT-IR spectra of CF and GQDs. FTIR spectrum of carbon fiber shows a straight line as it does not contains any functional groups. Oxygen containing functional groups such as carbonyl, carboxyl and hydroxyl groups were introduced to the edges of the monolayer graphene as shown in the FT-IR spectrum.