Supporting Information

Primary mouse hepatocytes imaging by multiphoton luminescent graphene quantum dots

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Experimental

Materials. Graphite powder was purchased from Bay Carbon, Inc. (SP-1 graphite powder). Potassium sodium tartrate was purchased from Sigma – Aldrich.

Synthesis Procedure. The overall processes of the proposed synthetic route for GQDs were previously reported in ref 20. Briefly, a potassium sodium tartrate with graphite was used for graphite intercalation compounds (GICs) at 250°C and the prepared GICs were exfoliated in water for breaking the graphene flakes. The abrupt reduction between the GICs and water not only exfoliates the graphene layers but also breaks the graphene flakes into GQDs.

Characterization. Morphology of GQDs was analysed using an atomic force microscope (AFM, SPA400, SⅡ, Japan) in tapping mode under ambient conditions. UV-Vis spectra (UV-3101PC spectrometer), fluorescence spectra (Perkin-Elmer LS 55 luminescence spectrometer), X-ray photoelectron spectroscopy (XPS, Sigma Probe, AlKα), transmission electron microscopy (TEM, Tecnai G2 F30) analyses were conducted. TEM samples were prepared by drying a droplet of the GQDs suspensions on a carbon grid. The FT-IR spectrum was measured using a FT-IR-4100 type-A FT-IR spectrometer with pure KBr as the background from 1000 and 3000 cm⁻¹. All the luminescent data were obtained by handmade setup using the precision cells made of quartz suprasil. The photoluminescence (PL) measurements, such as excitation wavelengths-dependent PL behaviors, were carried out using a 325 nm He-Cd continues-wave (CW) laser, a monochromatic light from a 300 W-xenon lamp, and UV spectrometers (Maya2000, Ocean Optics, USA) as a PL detector at room temperature. The PL excitations were measured by monochromatic light from a 300 W Xenon lamp and a high-sensitive photomultiplier tube as a PL detector. In order to elucidate the recombination dynamics, we carried out time-resolved PL experiments.
Characterization of GQDs

The high-resolution transmission electron microscopy (HRTEM) images of the GQDs are shown in Fig. S1a. The TEM image shows that the resultant GQDs has diameters of less than 10 nm, and the GQDs show a high crystalline structure with lattice parameter of ~ 0.21 nm, consistent with previous reports. The AFM images shown in Fig S1b present the topologic morphology of the GQDs, and show that the majority fraction is made up of the ~ 1.0 nm thick GQDs. Also, from the Fourier transform infrared spectroscopy (FT-IR) analysis of the GQDs and the tartrate salts (Fig. S1c), characteristic peaks related to C-H band (2923 cm$^{-1}$), C=O/COOH band (1724 cm$^{-1}$), and the -OH band (1379 cm$^{-1}$) are observed in both samples, while the epoxide band at 1032 cm$^{-1}$ is completely absent in the GQDs. These results strongly suggest that the GQDs fabricated by GICs have low surface defects and edge functional groups.

Figure S1. Characterization of GQDs a. HRTEM image of GQDs. b. AFM image of GQDs. c. FT-IR spectra of GQDs.
Photoluminescence characteristics of GQDs

**Single photon excitation.** As the excitation wavelength increases from 260 nm to 440 nm, the PL peaks redshift with a maximum intensity at ~435 nm (Figure S2a). Figure S2b shows the luminescence decay profile of the blue GQDs. The decay profile is fitted by double exponential with lifetimes of ~2.10 ns and ~7.88 ns. Two values of lifetime are ascribed to the different decay channel, intrinsic and extrinsic, of GQDs. We also measured the quantum yields (QYs) by using absolute PL QYs measurement system. The QYs of GQDs excited by 310 nm at room temperature was about 4.0% (Fig. S2c). The inset of Figure S1c shows the UV illuminated GODs dispersed in the water.

![Figure S2. Optical properties of the GQDs. a. PL spectra of the GQDs at different excitation wavelengths. b. TRPL decay profile of the GQDs recorded at room temperature. c. Quantum yields of GQDs. Inset: Emission images of GQDs dispersed in water.](image)

Figure S3 shows the variation of integrated PL intensity depending on the excitation power at different excitation wavelengths. All PL were measured from aqueous GQDs in water and the
excitation wavelength was changed from 700 nm to 875 nm. The increment of integral PL intensities vs. excitation power were fitted by straight lines for all cases with varying slope values between 1.6 and 2.8.

![Graph showing integral PL intensities versus excitation power](image)

**Figure S3.** The integral multi photon excited PL intensities versus the excitation power (plotted in a logarithmic scale) under various excitation wavelengths from 700 nm to 875 nm. The slope value of the integral PL intensities versus the excitation power by fitting with straight lines.

**Primary mouse hepatocytes imaging by multi-photon excitation**

**Cytotoxicity evaluation.** The cells were cultured and maintained in DMEM low glucose medium (WelGene) containing 1g/mL D-glucose, 4 mM L-glutamine, and 110 mg/mL sodium pyruvate, with 10% fetal bovine serum (FBS), 100 IU mL-1 penicillin and 100 µg/ml streptomycin. Freshly isolated primary mouse hepatocytes were plated at 3×10⁵ cells per well on 6 well plates, and were left to adhere overnight. Different concentrations of GQDs (0-100 µg/mL) in culture media were added to each well in duplicate. The cells were then incubated for 24 hours (Fig. S4a). At the end of the study, cell soups were collected and performed LDH level test using by VetTest Chemistry analyzer according to manufacturer’s instruction. But comparatively, CCl₄ and EtOH (mouse hepatocytes + 4hr fasting + DMEM (1% Anti) + EtOH or CCl₄ for 24hr : duplication), which is well known as liver injury factors, treated hepatocytes as a positive control release significantly severe LDH level (Fig. S4b-d).
Figure S4. Positive control. a. Contrast image of hepatocyte after treated GQDs for 24 hours. b and c. LDH test for the Positive controlled hepatocytes by EtOH (b) and CCl₄ (c). d. Table for the values from positive control of EtOH and CCl₄.

**Cell imaging.** For imaging, $3\times10^5$ cells were plated in each well of a 6 well plate with 2 mL culture medium. After overnight culture, 50 µg/mL GQDs was added in culture medium and incubated in regular cell culture conditions. After 24 hours of culture, medium with GQDs was removed from the cells and washed two times with 1ml 1X filtered PBS. Cells were fixed using 1% paraformaldehyde and mounted with Vectashield antifade mounting media with DAPI (Vector Laboratories, Inc., CA) (main Fig. 3c). For the concentration dependent cell imaging, the cytotoxicity tested samples were used. Cellular imaging was done using a LEICA DMI 4000B microscope equipped with the DFC450C camera. LEICA CTR 4000 lamp was used as the fluorescence light source. For the multi-photon imaging at 790 nm, Zeiss LSM 510 META confocal microscope was used. Figure S5 shows high magnified images of hepatocytes incubated with 50 µg/mL GQDs measured by single photon fluorescence.
Figure S5. Single photon image of primary mouse hepatocytes: Phase contrast image (top left), stained by DAPI (top right) and GQDs’ fluorescence (bottom left) images, and merged image (bottom right)
For the photo-bleaching test, we repeated multi-photon imaging continuously 7 times at the same position (Fig. S6a) using excitation wavelength of 790 nm. Also, we captured cell images with different excitation wavelengths (543 nm and 633 nm) as shown in Fig. S6b after series multi-photon imaging.

For the durability test, multi-photon imaging was conducted right after and two week after 24 hours of culture with GQDs which are shown in Fig. S7.

Figure S6. Photo-bleaching test. a. Continuously 7 times repeated multi-photon imaging with excitation wavelength of 790 nm (4: 633 nm) b. Fluorescence images with different excitation wavelengths (790, 543, and 633 nm) after series measurement.

Figure S7. Durability test. Hepatocyte images right after and two weeks after 24 hours incubation with GQDs.