Supplementary Material (ESI) for Journal of Materials Chemistry B
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Electronic Supporting Information

One-step preparation of nitrogen-doped graphene quantum dots from oxidized debris of graphene oxide

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Experimental Section

1. Preparation of N-GQDs

GO was prepared from native graphite flake according to the modified Hummer's method.[1, 2] N-GQDs are obtained by hydrothermal treatment of GO in the present of ammonia. In a typical procedure, 30 ml as-prepared GO water dispersion (1 mg/mL) was mixed with 0.3 mL of ammonia solution (28 wt% in water) in a glass vial. After stirring for 10 min, the mixture was transferred to a Teflon lined autoclave and heated at 180 °C for 12 h. After cooling to room temperature, the black precipitated (RGO) were filtered out using a mixed cellulose ester membrane with 25 nm pores (Millipore). The light yellow supernatant was dialyzed in a 3000 Da dialysis bag against deionized water for 2 days to remove excess ammonia. The resultant light yellow solution was freeze-dried, and a light yellow powder of N-GQDs was obtained.

2. Characterization methods

High-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL-2010 electron microscope operating at 200 kV. X-ray diffraction (XRD) patterns were obtained from a MSAL-XD2 X-ray diffractometer with Cu Ka radiation (40 kV, 20 mA, λ = 1.54051 Å). The Raman spectrum of as-prepared samples was recorded at ambient temperature on RenishawRM 2000 with an argon-ion laser at an excitation wavelength of 785 nm. The Fourier transform infrared spectroscopy (FTIR) spectra were measured by an EQUINOX 55 (Bruker) spectrometer with the KBr pellet technique ranging from 500 to 4000 cm^{-1}. AFM image was taken in tapping mode with the SPM Dimension 3100 from Veeco. X-ray photo-electron spectroscopy (AXIS ULTRA DLD, Kratos) was used to investigate the functional groups present of the surface of the N-GQDs. A Cary 5000 UV-visible-near-infrared (NIR) spectrometer (Varian) was used to follow the absorbance of the GQDs. The fluorescence spectra of the N-GQDs were measured with a fluorescence spectrometer F-4500 (Hitachi, Japan).

3. In vitro cytotoxicity against HeLa cells

In 96-well plates, 100 μL supspension of HeLa cells (5×10^4 cells/mL) in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (DMEM) were added to per well and incubated in a 5% CO_2 humidified incubator at 37 °C for 24 h. The GQDs were introduced into the wells in a concentration of 12.5, 25, 50, and 100 μg/mL and the four wells incubated for another 24, 48, 72 h, respectively. The medium was removed and cells were washed with phosphate-buffered saline. Then, 20 μL of 5 mg/mL MTT solution was added to each cell well. The 96-well plates were further incubated for 4 h, followed by removing the culture medium with MTT, and then 200 μL of DMSO was added. The resulting mixture was

shaken for 10 min at room temperature. The optical density of the mixtures at 490 nm was measured. Cell viability was expressed as percentage of absorbance relative to control, the control was obtained in the absence of N-GQDs. Experiments were performed in triplicates, with nine replicate wells for each sample and control per assay.

4. Fluorescence Imaging Experiments

HeLa cells were seeded in each well of a confocal dish (Coverglass-Bottom Dish) and cultured at 37 °C for 24 h. An aqueous solution of the N-GQDs (0.1 mg/mL) was passed through a 0.2 μm sterile filter membrane. The filtered fluorescent suspension (40-60 μL) was mixed with the culture medium (200 μL) and then added to three wells of the confocal dish (the fourth used as a control) in which the HeLa cells were grown. After an incubation of 6 h, the medium was removed and the cells were washed thoroughly three times with PBS (500 μL each time) and kept in PBS for the optical imaging. Cellular uptake of N-GQDs by HeLa cells tracked *via* confocal microscopy and the emission was measured over the range of 450-550 nm, λ_{ex} = 405 nm.

5. Quantum Yield Measurements

The quantum yield (Q) of N-GQDs was calculated with the following equation. Quinine sulfate in 0.1 M H_2SO_4 (Q = 0.54 at 340 nm) was chose as a standard. Since Q is the quantum yield, I is the measured integrated emission intensity, n is the refractive index, and A is the optical density. The subscript R refers to the reference fluorophore of known quantum yield.

$$Q = Q_R \frac{I}{I_R} \frac{A_R}{A} \frac{n^2}{n_R^2}$$

Table S1 Quantum yield of the N-GQDs

Sample	Intergrated emission intensity (I)	Abs. At 340nm (A)	Refractive index of solvent (n)	Quantum yield at 360nm (Q)
Quinine sulfate	41601.1	0.068	1.33	0.54 (known)
N-GQDs sample	14492.4	0.052	1.33	0.246

Table S2 O/C and N/C atomic ratio of GO, RGO and N-GQDs from the XPS analysis.

Sample	O/C (%)	N/C (%)
GO	39.22	1.32
RGO	12.09	10.45
N-GQDs	66.23	17.88

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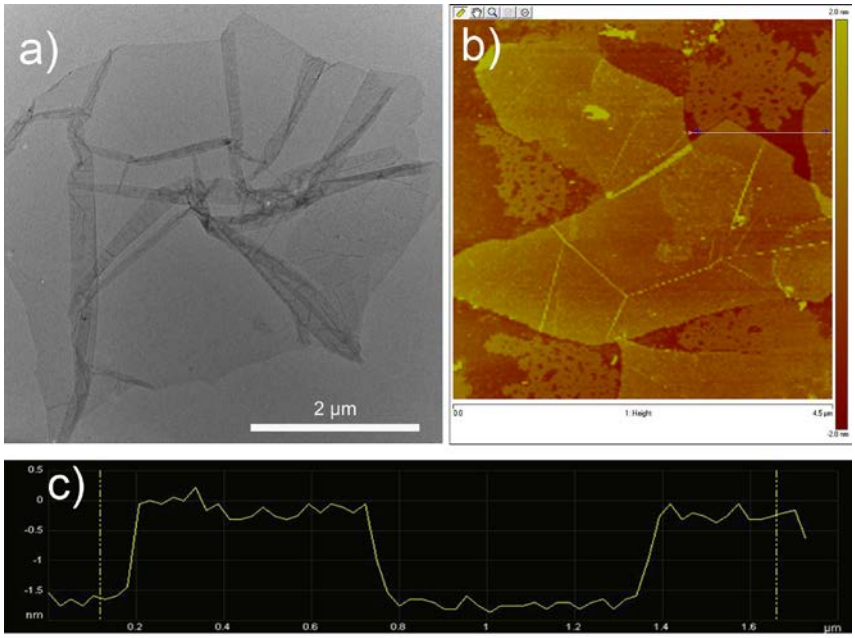

Fig. S1 TEM (a) and AFM (b) images of GO. (c) Height profile of GO corresponding to the AFM image.

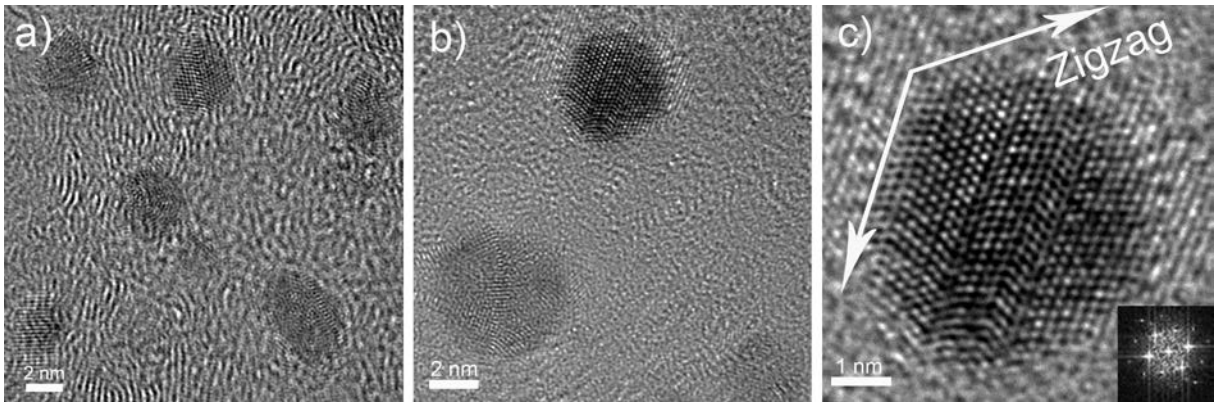

Fig. S2 HRTEM images of N-GQDs at different magnification. In set of (c) is FFT image of N-GQDs.

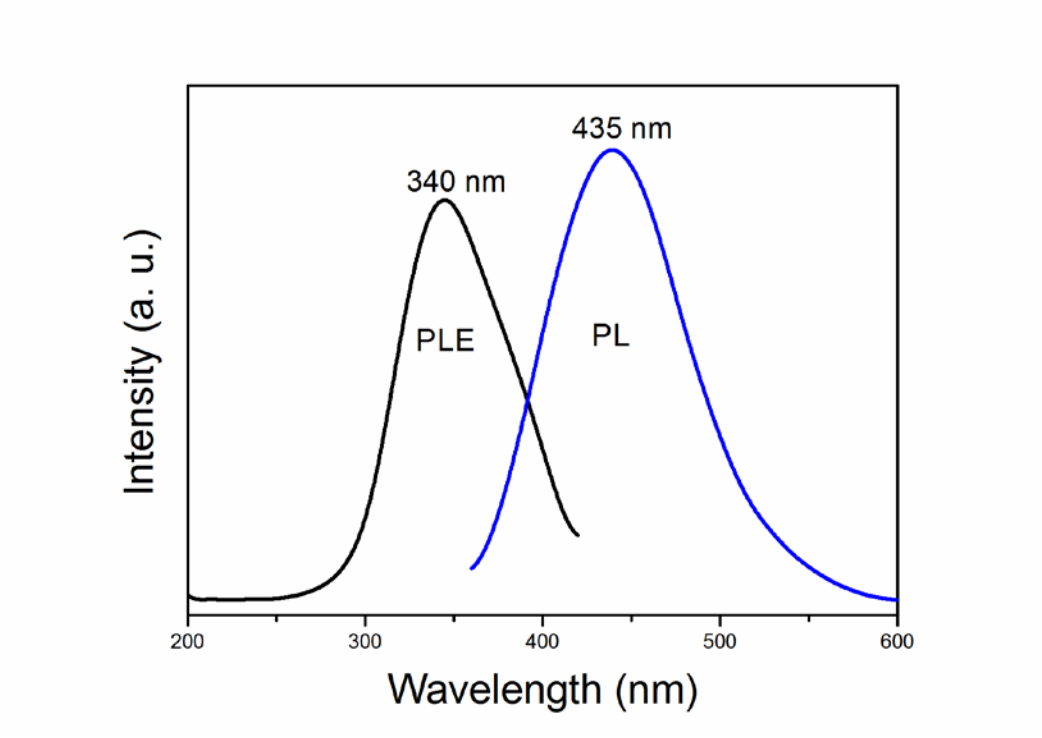

Fig. S3 PLE spectrum of N-GQDs with the detection wavelength of 435 nm and PL spectrum excited at 340 nm.

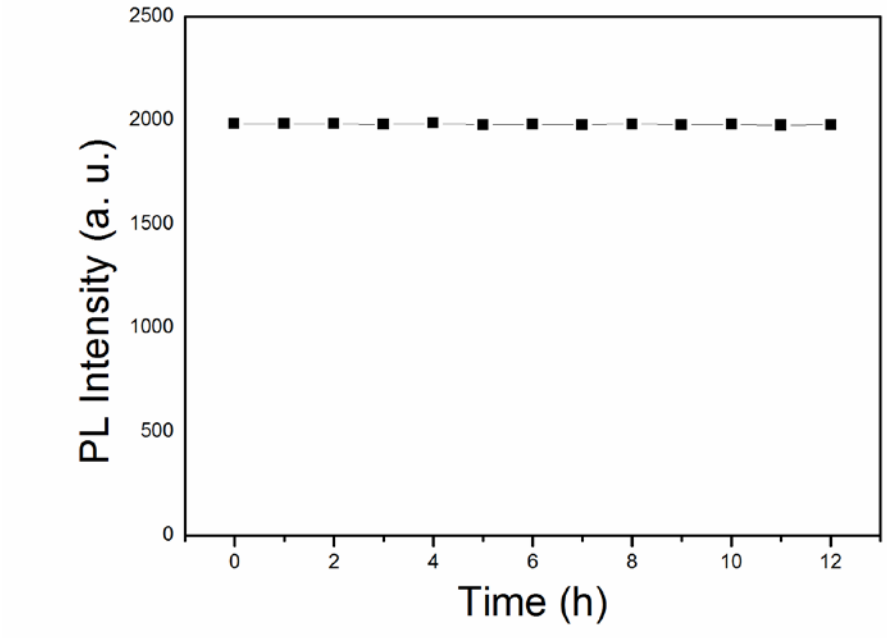

Fig. S4 Photostability test of the N-GQDs in a fluorescence spectrophotometer with a 150 W Xe lamp under 320nm excitation.

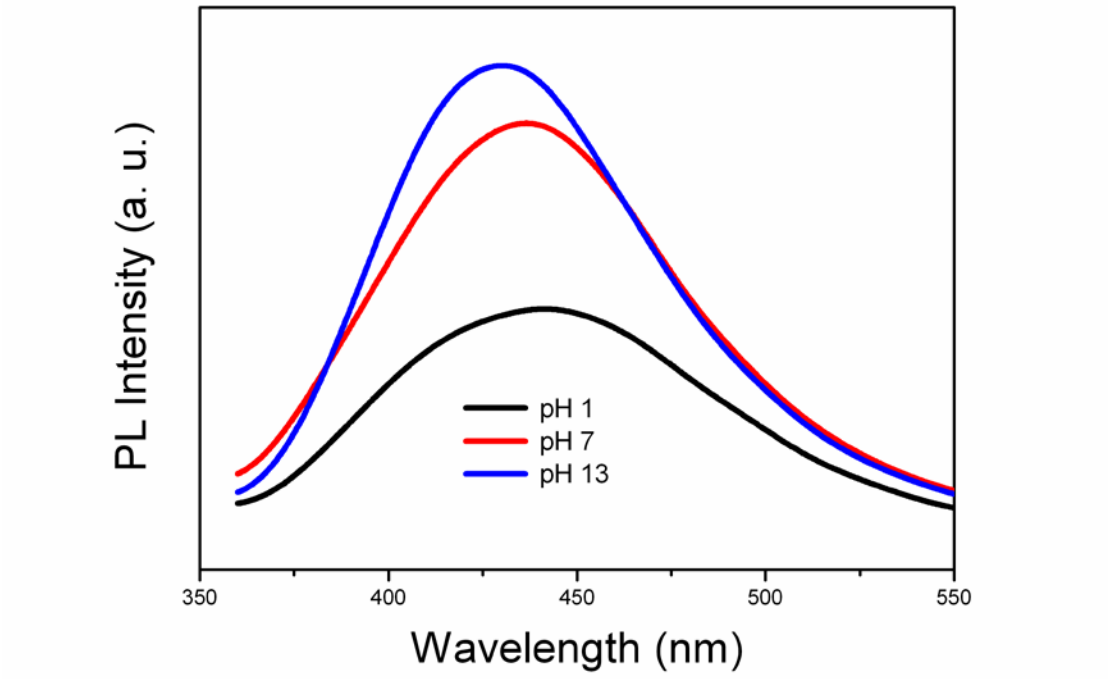

Fig. S5 Effect of pH value on the PL intensity of N-GQDs at various pHs (λ_{ex} = 340 nm).

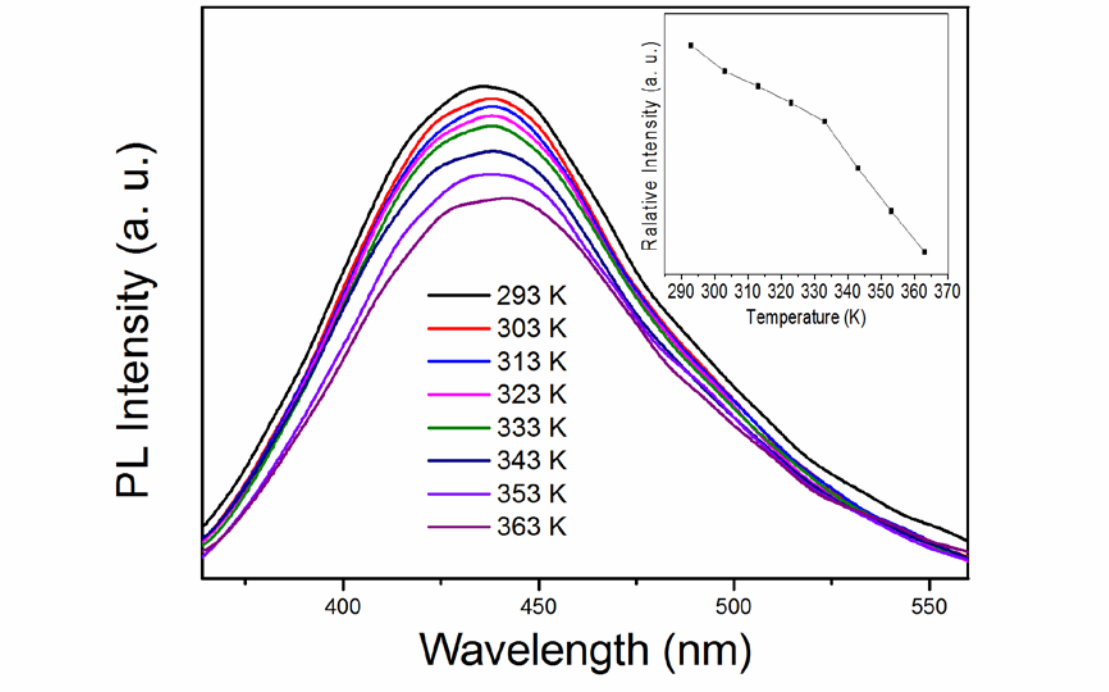

Fig. S6 Effect of temperature on the PL intensity of N-GQDs (λ_{ex} = 340 nm).

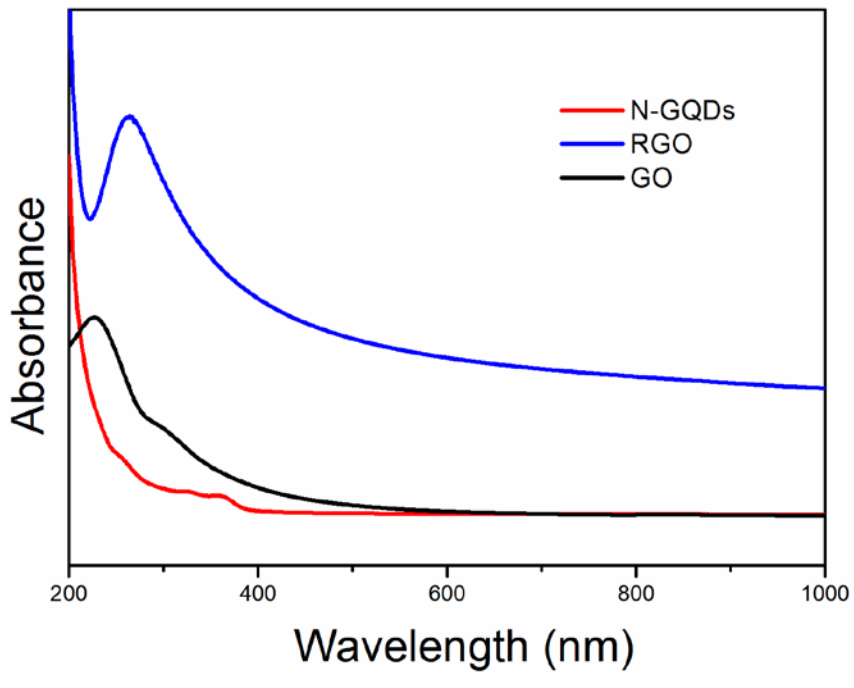

Fig. S7 UV-vis spectra of GO, RGO and N-GQDs.

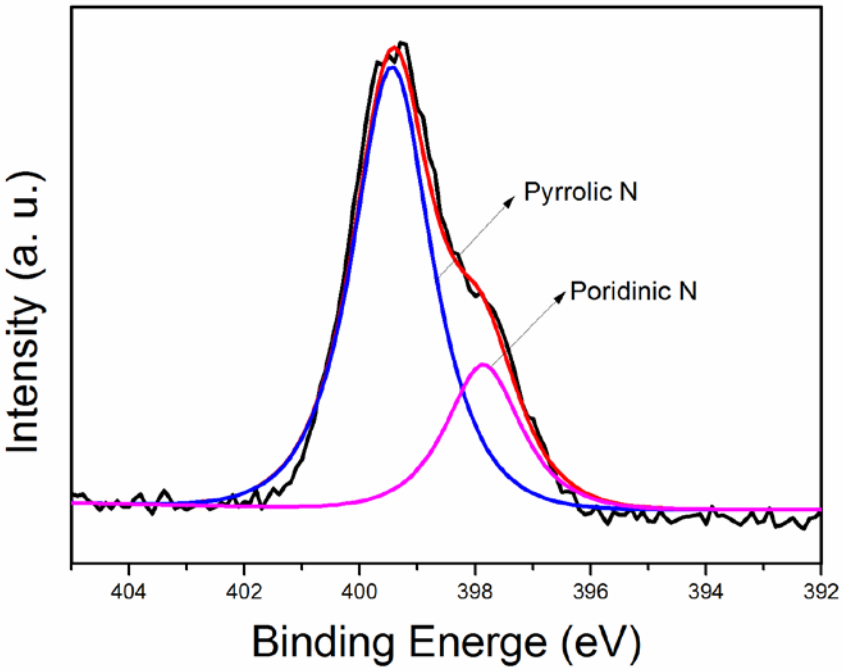

Fig. S8. N1s signals of N-GQDs.

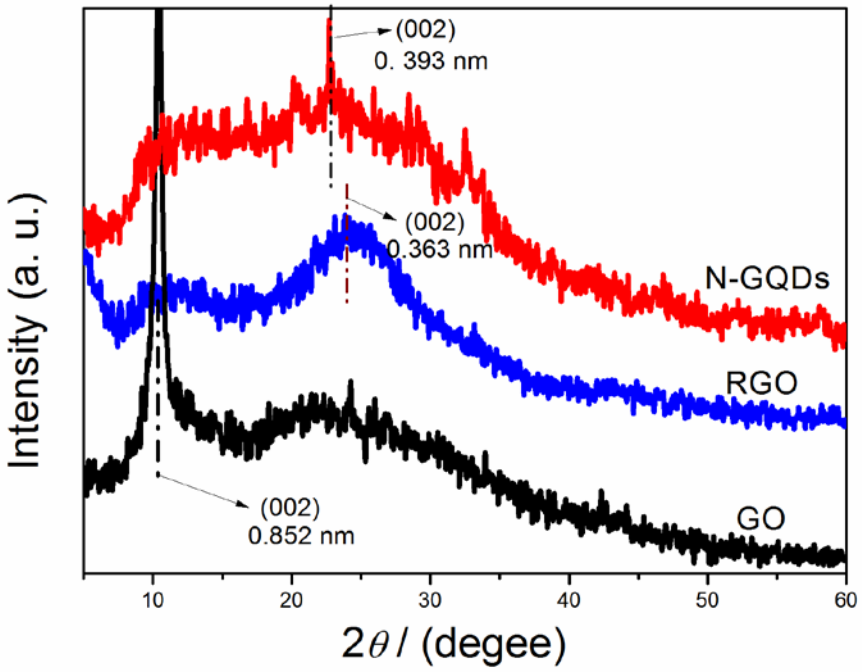

Fig. S9. XRD patterns of GO, RGO, and N-GQDs.

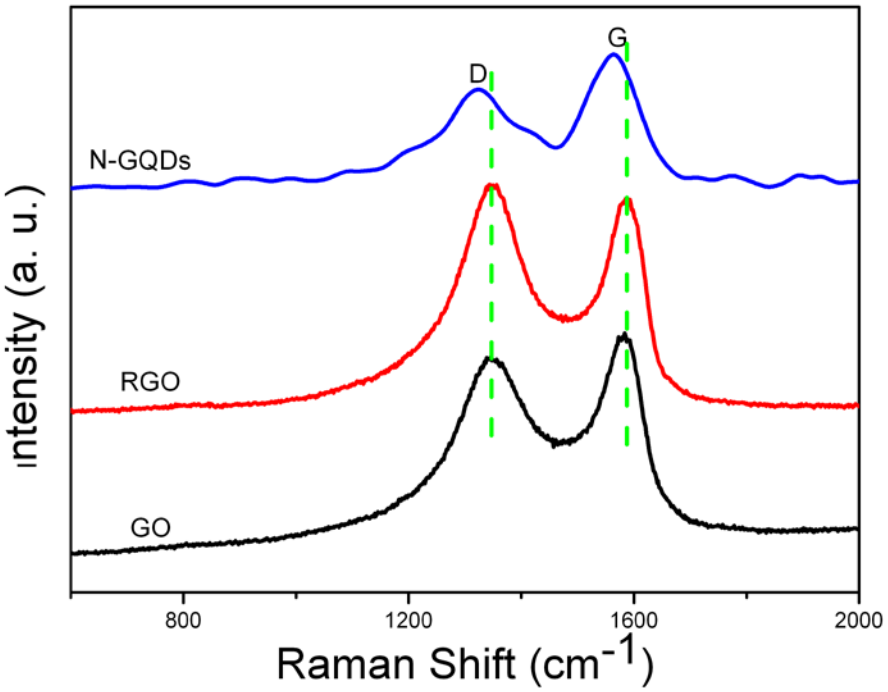

Fig. S10 Raman spectra of GO, RGO and N-GQDs with 514 nm laser excitation.

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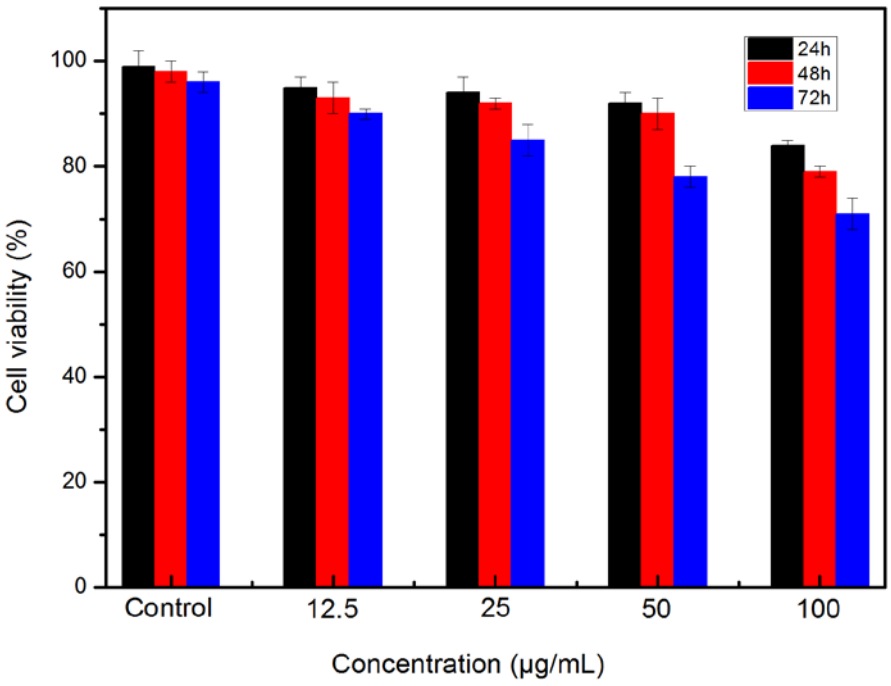

Fig. S11 Cytotoxicity evaluations test of HeLa cells with different concentrations of N-GQDs after 24 h, 48 h and 72 h incubation.

Reference

1 W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.*, 1958, **80**, 1339-1339.

2 N. I. Kovtyukhova, P. J. Ollivier, B. R. Martin, T. E. Mallouk, S. A. Chizhik, E. V. Buzaneva and A. D. Gorchinskiy, *Chem. Mater.*, 1999, **11**, 771-778.