Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 647–650

Fabrication of carbon quantum dots *via* ball milling and their application to bioimaging

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Experimental Details

Conductive carbon black, Ketjen black (KJ) (diameter: 20-30 nm), and sodium carbonate were purchased from Lion Specialty Chemicals Co, Ltd., and J. T. Baker., respectively. Quinine sulfate salt monohydrate for the calibration of quantum yield of CQDs was purchased from Sigma-Aldrich. Nylon filter membranes with a pore size of 0.22 μm was purchased from Millpore. A dialysis bag with a molecular weight cut-off of 3500 Da was obtained from Orange Scientific.

The CQDs were fabricated as follows. KJ carbon black (0.8 g), sodium carbonate (Na_2CO_3, 4.9 g) and zirconia balls (100 μm, 145 g) were placed in a 125-ml grinding jar. The process was conducted using a planetary miller (Retsch PM 100) with a rotation speed at 500 rpm for 24 h. After the process, deionized water was poured into the jar to form a CQD dispersion solution. The solution was centrifuged (5000 rpm) and the supernatant was then obtained and filtered (0.22 μm filter). The filtrate was dialyzed for a period of 24 h. The processed dialysate was freeze-dried to obtain the CQDs.

Field emission scanning electron microscope (FE-SEM, Hitachi S-4800-I) and transmission electron microscopy (TEM, JEOL JEM-F200) were used to observe the morphology of the raw materials and the CQDs. MicroRaman spectrometer (Raman, Horiba XploRA), Fourier transfer infrared spectrometer (FTIR, Bruker VERTEX 70v) and high resolution x-ray photoelectron spectrometer (XPS, UPLC Hybrid Quantera) were employed to investigate the chemical binding of the CQDs. The absorbance and fluorescence spectra of CQDs were obtained by a spectrophotometer (Shimadzu UV-2600, 365 nm) and a photoluminescence (PL, Hitachi F4500), respectively. The quantum yield of the CQDs was calculated based on the PL spectrum of quinine sulfate.

For the cell viability study, typically, HeLa cells (6×10^3 cells/well) and the CQDs with various concentrations (0, 50, 100, 200, 400, 800 and 1000 μg/mL) were supplemented in a 100 μL of minimum essential medium containing Earle's salts and l-glutamine (MEM, Corning, USA) with 10 % fetal bovine serum, 1 % amino acid, sodium pyruvate, and antibiotic-antimitotic to each well in 96-well flat plates at 37°C for 24 h. After the incubation, the medium was removed and 110 μL thiazolyl blue tetrazolium bromide (often called MTT) (5 mg/mL) was added to each well for 3 h incubation. Then, the culture medium was removed and then 50 μL dimethyl sulfoxide was added. The absorbance of the colored suspension was examed with a 570 nm wavelength radiation by a microplate reader. The cell viability was calculated using following equation.[S1]

$$\text{Cell viability (\%)} = \frac{O.D.\ of\ CQD\ treaded}{O.D.\ of\ control}\ X\ 100\% \qquad (1)$$

where *O.D. of CQD* and *O.D. of control* are the absorbance of the cells incubated with and without the CQDs, respectively.

For the imaging study, HeLa cells were seeded in a 24-well flat plate (1 mL/well) and cultured in minimum essential medium containing Earle's salts and l-glutamine (MEM, Corning, USA) with 10 % fetal bovine serum, 1 % amino acid, sodium pyruvate, and antibiotic-antimitotic at 37 °C in a humidified CO_2 atmosphere for 24 h. CQDs (200 μg/mL) were added in the plates for a further 24 h incubation. After the incubation, the medium was discarded and the cells were rinsed three times with phosphate buffer solution (10 mM, pH 7.4, PBS). Formalin (4 %) in PBS was then added in the cell for 15 min at ambient temperature. The cell was rinsed by PBS to remove the excess formalin before imaging experiments.

Results and discussion

Figure S1(a) and S1(b) show the FE-SEM images of KJ carbon black. As can be seen, the shape of the particles is irregular and the diameters are in the range of 20-30 nm. No serious aggregation was observed. Figure S1(c) and S1(d) show the TEM images of the KJ carbon black. The image reveals that the particles were connected together partially and only a few individual particles were observed. The image in Figure S1(d) suggests that the particle has a short-rang order of graphitic structure indicating the existence of many defects in the edge of the carbon black.

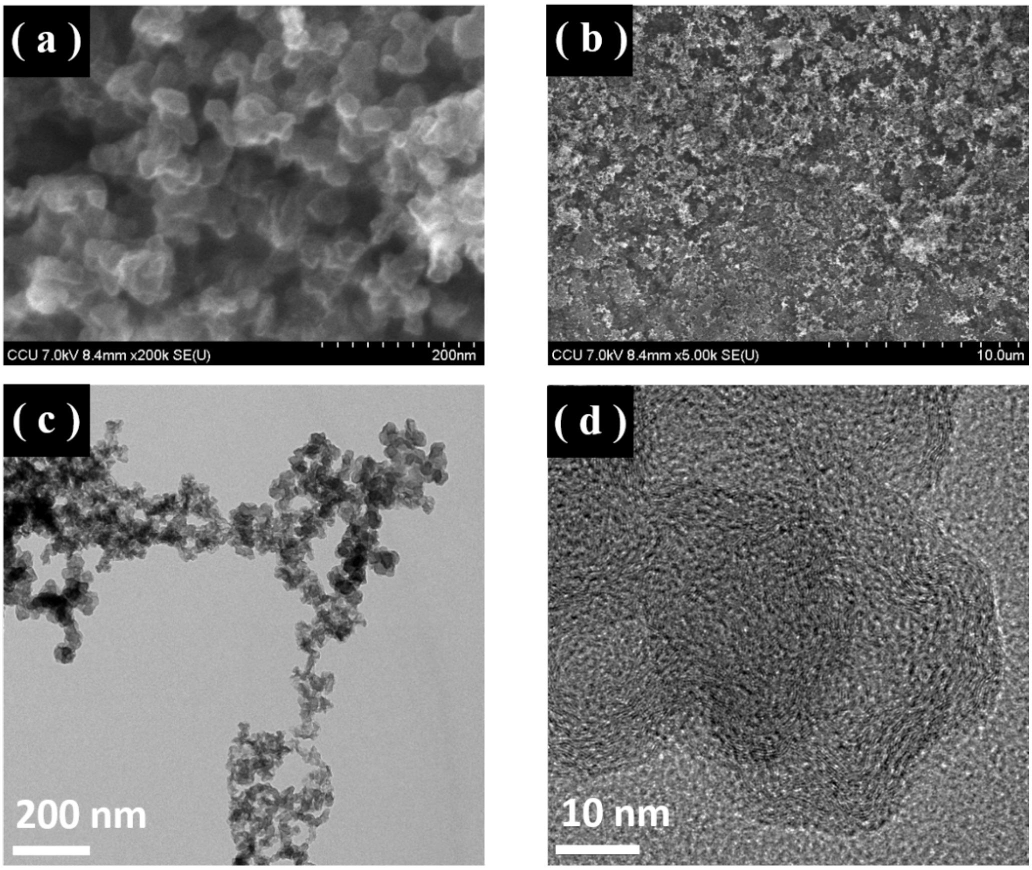

Figure S1 KJ carbon black. SEM images of (a) high magnification and (b) low magnification. TEM images of (c) low magnification and (d) high magnification.

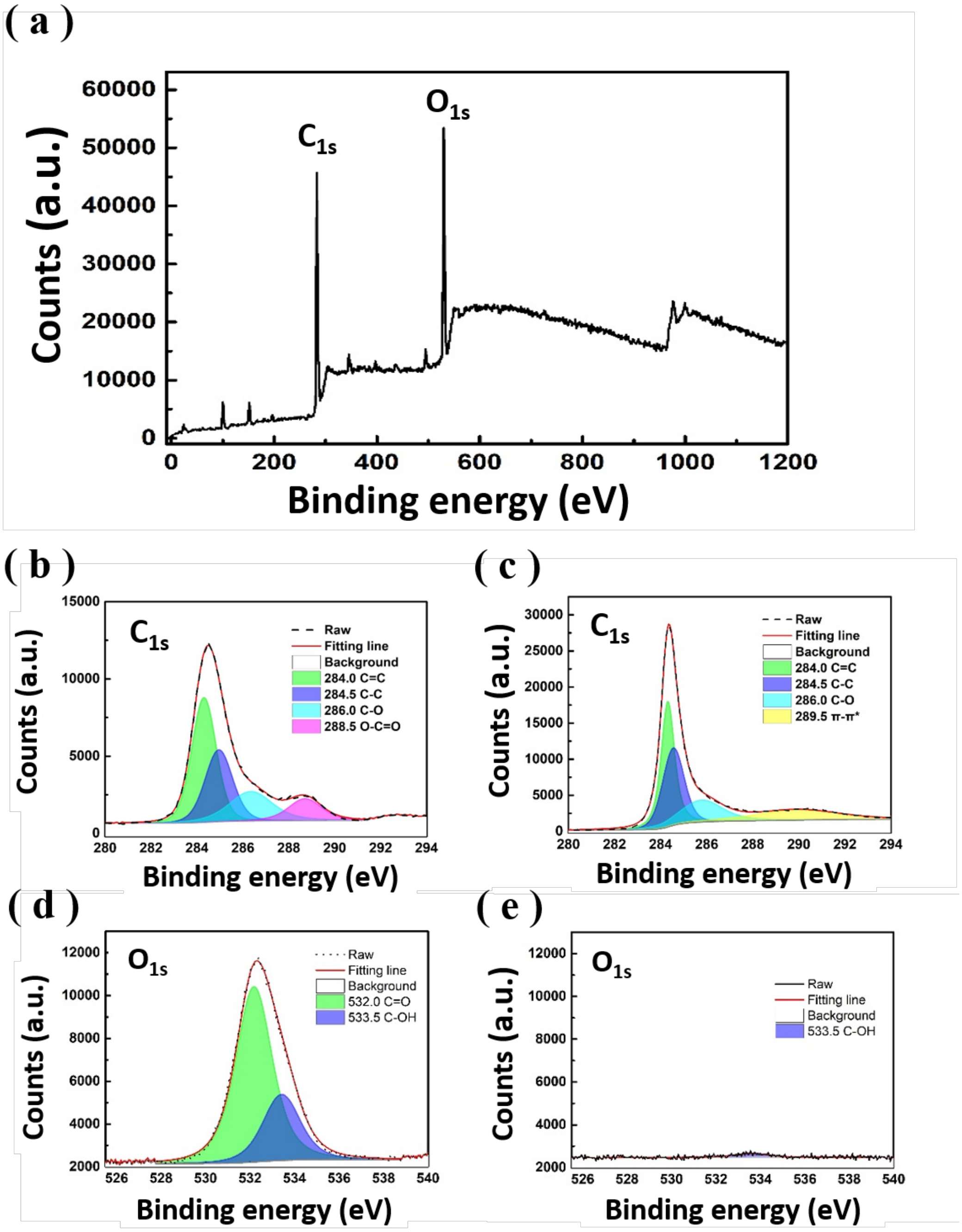

Figure S2 XPS of KJ carbon black and CQDs obtained using Na_2CO_3 as a secondary abrasive medium. (a) Survey spectrum of CQDs, (b) C_{1s} spectrum of CQDs, (c) C_{1s} spectrum of KJ carbon black, (d) O_{1s} spectrum of CQDs and (e) O_{1s} spectrum of KJ carbon black.

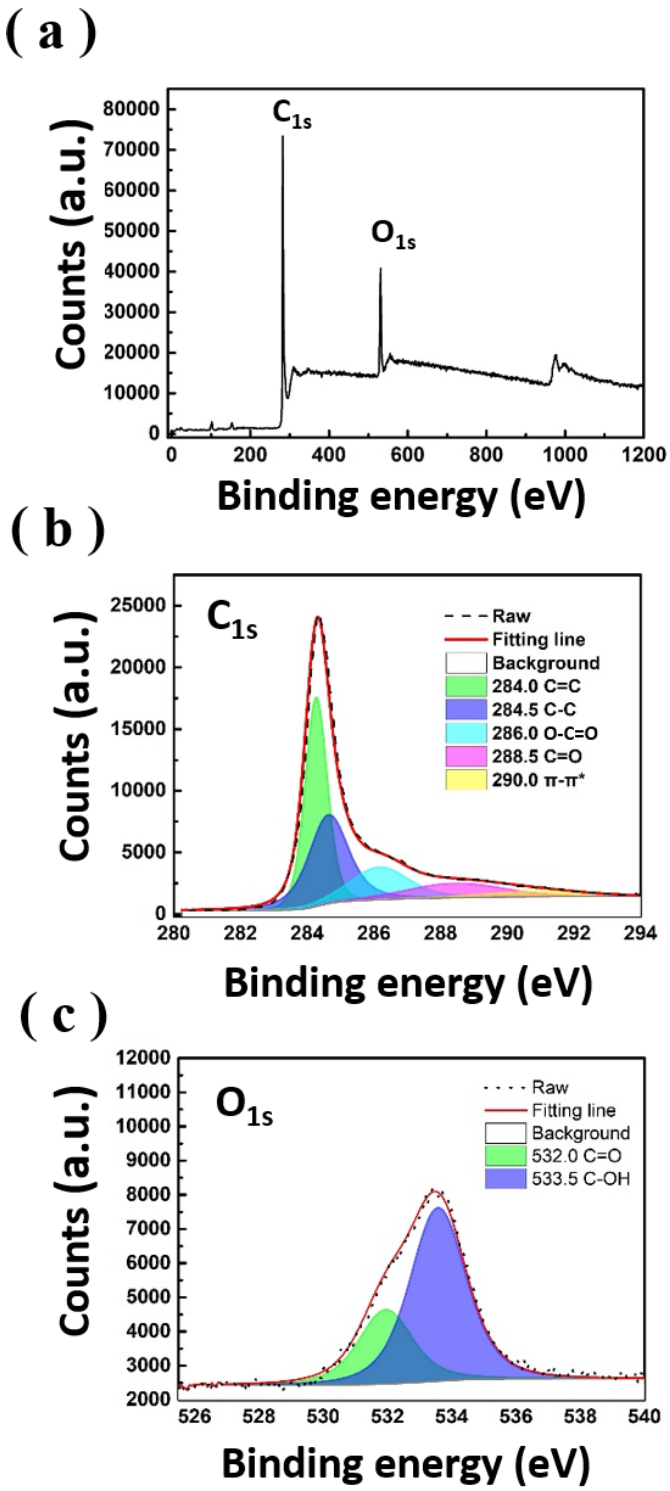

Figure S3 XPS of CQDs obtained without using Na_2CO_3 as a secondary abrasive medium. (a) Survey spectrum, (b) C_{1s} spectrum and (c) O_{1s} spectrum.

Figure S4(a) demonstrates the Raman spectra of KJ carbon black and the CQDs. The I_D/I_G ratios of conductive KJ carbon black and CQDs are 1.34 and 1.0, respectively, indicating that the graphitization degree was increased after ball milling.[S2] Figure S4(b) shows the FTIR spectra of the CQDs and KJ carbon black. The peaks of C-OH, C-O and C=C are observed at 3400-3260 cm^{-1}, 1380 cm^{-1}, and 1620-1590 cm^{-1}, respectively. The intensity of C-O bond in the CQD spectrum is relatively higher than that in the KJ carbon black indicating the CQDs have more oxygen functional groups in comparison to the KJ carbon black. In addition, the intensity of the peak representing C=C bond in CQD spectrum is higher than that in the KJ spectrum indicating the graphitization of the CQD is higher than that of KJ carbon black. The result agrees well with the result of Raman spectra.

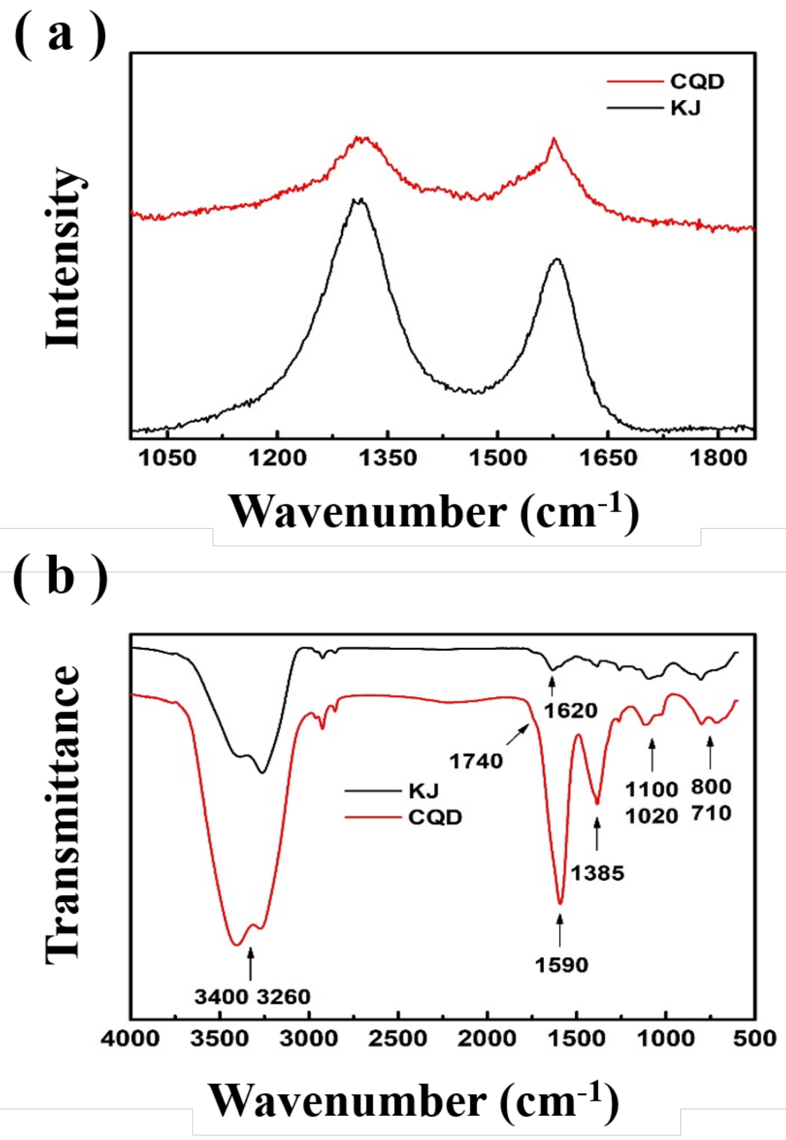

Figure S4 (a) Raman spectra of KJ carbon black and CQDs and (b) FTIR spectra of KJ carbon black and CQDs.

References

S1 M. Li, T. Chen, J. J. Gooding and J. J. A. s. Liu, *ACS Sensors*, 2019, **4**, 1732-1748.

S2 T. Xing, T. Ramireddy, L. H. Li, D. Gunzelmann, H. Zeng, W. Qi, S. Zhou and Y. J. P. C. C. P. Chen, *Phys. Chem. Chem. Phys.*, 2015, **17**, 5084-5089.