A novel rapid and green synthesis of highly luminescence carbon dots with good biocompatibility for cell imaging

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Characterization of the C-dots

High-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL-2010 electron microscope operating at 200 kV. Atomic force microscopy (AFM) image was obtained with a Micronano New Spm atomic force microscope (zhuolun, China). X-ray diffraction (XRD) patterns were obtained from a Rigaku D/max-2550VB/PC X-ray diffractometer with Cu Ka radiation (40 kV, 450 MA). The Fourier transform infrared spectroscopy (FTIR) spectra were measured by an NICOLET iS10 (Thermo) spectrometer with the KBr pellet technique ranging from 500 to 4000 cm^{-1}. UV2450 spectrophotometer (Hitachi, Japan) was used to determine the absorbance of the C-dots. The fluorescence spectra of the CNPs were measured with a F900 fluorescence spectrometer (Edinburgh, UK), with a slit width of 2 nm and 2 nm for excitation and emission, respectively. Cell imaging with C-dots was obtained by the laser scanning confocal microscopy (LSCM, Leica DM6000 CS).

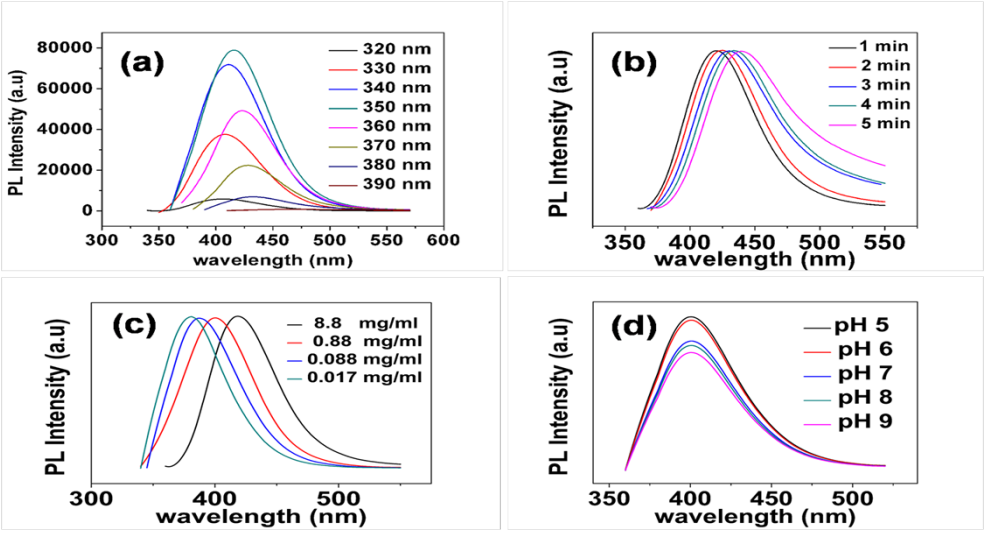

Figure S1. (a) emission spectra of C-dots at excitation wavelength progressively increasing from 320nm to 390nm, (b) Normalized optimal emission spectra of C-dots prepared with different time(concentration of C-dots: 0.4mg/ml), (c) Normalized optimal emission spectra of C-dots of different concentrations (heating time: 1min), (d) PL spectra at 340nm excitation within various pH(heating time 1min, 0.06mg/ml).

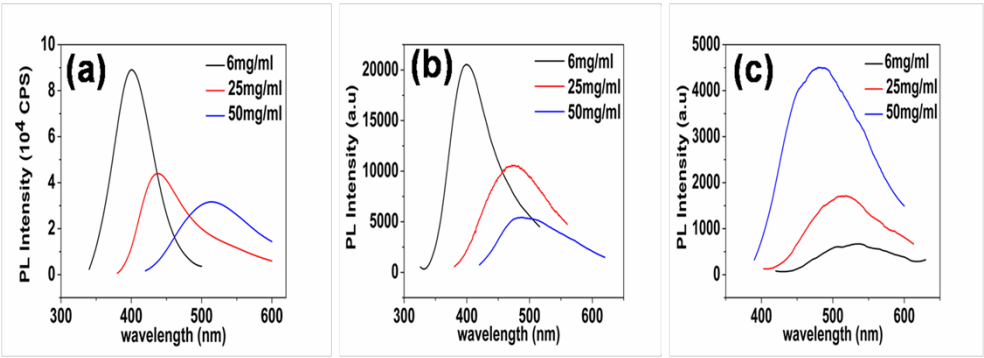

Figure S2. Emission spectra of C-dots made by (a) M-H, (b) H and (c) M methods with different concentration of ascorbic acid.

Plausible mechanism for the formation of C-dots and the origin of the fluorescence

Similarly to the hydrothermal approach, [1] we suggest that under the rapid,

simultaneous and homogeneous microwave heating presented here, the C-dots were synthesized through the "polymerization" and "carbonization" steps within a few minutes.

Since this M-H method involves no dialysis process, it may argue that this fluorescence possibly result from the molecular precursors that remaining with the C-dots after centrifugation. [2] However, there are two reasons to ascribe the fluorescence to C-dots. Firstly, no fluorescent model amides would form because there is no nitrogen element exists in ascorbic acid molecular, and second, the ascorbic acid molecular is non-fluorescent.

Quantum yield measurement

After cross-calibrated the standard quinine sulfate with Rhodamine 6G, fluorescence quantum yield (QY) of C-dots was measured by comparing the integrated photoluminescence intensities (excited at 320 nm) and the absorbance value (at 320 nm) using quinine sulfate in 0.1 M H_2SO_4 (Φ_F=0.54) as standard. Different concentrations of the C-dots and standards were made, all of which had absorbance less than 0.1 (to minimize the inner-filter effects) at their excitation wavelengths. The fluorescence spectra for the calculation of quantum yield should be corrected for nonlinear instrument response before the integration of their total intensities.

QY was calculated using the following equation:

$$\Phi_x = \Phi_s \left(\frac{\mathrm{Grad}_x}{\mathrm{Grad}_s}\right)\left(\frac{\eta_x^2}{\eta_s^2}\right)$$

Where the subscripts x and s denote test and standards, respectively, Φ is the quantum yield, η is the refractive index, and Grad is the gradient from the plot of

integrated fluorescence intensity *vs* absorbance.

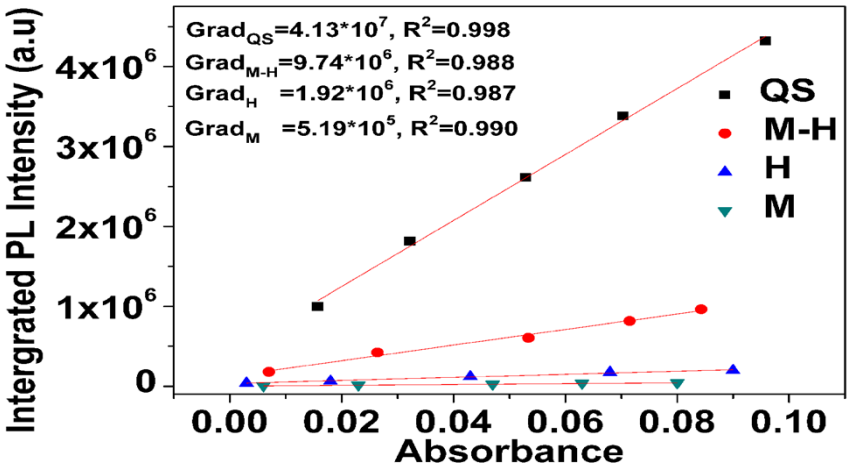

Figure S3. Fluorescence and Absorbance of the C-dots and Quinine Sulfate (QS)

In vitro cytotoxicity assay

90 µl suspension of BCap-37 cells cultured in Dulbecco's modified Eagles medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin (DMEM) were added to per well of 96-well plates(1×10^4 cells/well) and incubated in a 5% CO_2 humidified incubator at 37℃ for 24 h. The C-dots incubated with the same medium of cells' were introduced into the wells in a final concentration of 100, 200, 300, 400 and 500µg/ml and incubated for another 24h. After the medium was removed and cells were washed with phosphate-buffered saline, 20 µL of methylthiazolyldiphenyl-tetrazolium bromide (MTT) solution (5mg/ml) was added to per well and further incubated for 4h to allow violet colored formazan to form. After removing the culture medium with MTT, 150 µL of DMSO was added. Following shaking the resulting mixture for 10 min, the absorbance of the mixtures at 595 nm was measured with Bio-Rad model-680 microplate reader. Cell viability was obtained by calculating the percentage of absorbance relative to control sample which contained no

C-dots. Each sample and the reference had six replicate wells and experiments were performed in triplicates for mean values.

Confocal fluorescence microscopy image

2ml BCap-37 cells (maintained in Dulbecco's modified Eagles medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin (DMEM)) were seeded in a Cover glass-Bottom Dish and cultured at 37°C for 24 h. After passing through a 0.22 μm sterile filter membrane, An aqueous solution of the C-dots (5 mg/m, 100ul-400ul) was added to three wells of the confocal dish (the fourth used as a control) in which the cells were grown and then incubated for 24h. After the medium was removed and the cells were washed thoroughly two times with PBS (0.5ml each time), cellular image of C-dots labelled BCap-37 cells via confocal microscopy was measured under the excitation wavelength of 405 nm and 488nm.

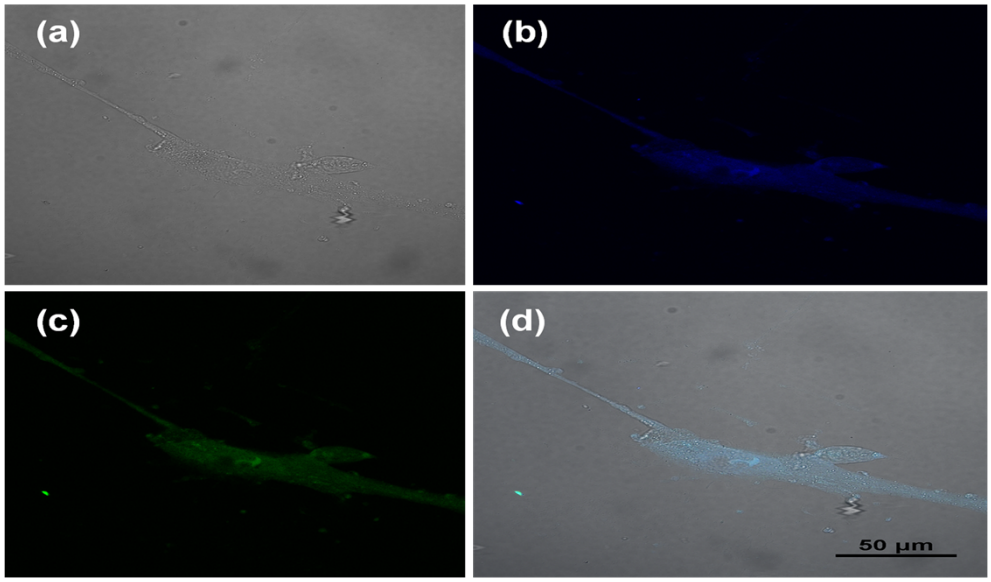

Figure S4. (a), (b) and (c) are confocal fluorescence microphotograph of carbon-dots labeled Hs-578 cells under bright field, 405nm excitation and 488nm excitation respectively, (d) overlay image of the above.

Ref:

1. X. Jia, J. Li and E. Wang, *Nanoscale*, 2012.
2. M. J. Krysmann, A. Kelarakis, P. Dallas and E. P. Giannelis, *Journal of the American Chemical Society*, 2011, **134**, 747-750.