

Multi-functional Fluorescent Carbon Dots with Antibacterial and Gene Delivery Properties

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Supplementary information

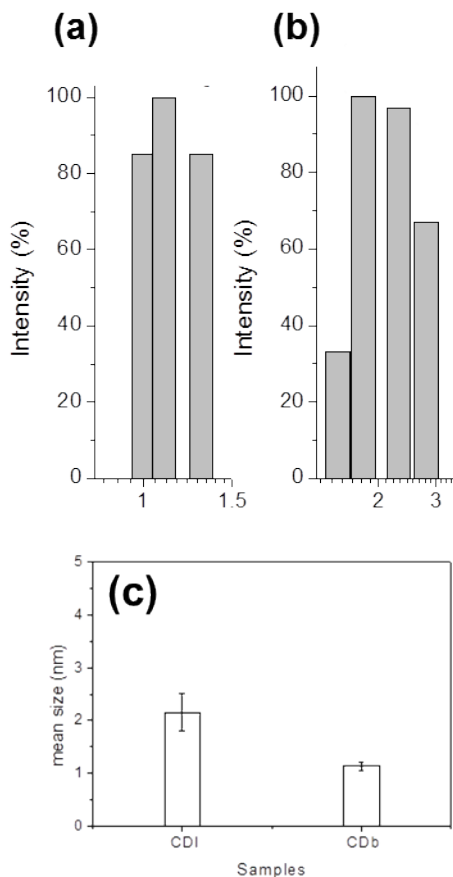


Fig. S1 (a) Size distribution of the the carbon dots passivated with branched PEI (1.0 g), CDb to the reaction. (b) Size distribution of the the carbon dots passivated with linear PEI (1.0 g), CDI, to the reaction. Glucose is kept constant at 0.3 g. (c) DLS data of carbon dots with standard deviation. PDI is 1.133 for CDI and 1.006 For CDb.

Fluorescence imaging of the carbon dots stained HEK293T cells was done with laser scanning confocal microscope equipped with an argon ion laser. C-dots were diluted to 10 nM in deionized water. Each 5 μ L aliquot was dropped onto a glass slide with fixed HEK293T cells, followed by drying in air. The samples were imaged under the 405 nm excitation and the image was taken at 500 nm. We did not have any difficulty in obtaining the fluorescent images.



Fig. S2 Fluorescent microscope image of HEK293T cells after incubation with CDs.

Materials and Methods

Synthesis of carbon dots

All the chemicals are used as received without further purification. In a typical synthesis, 0.3 g of glucose and 1.0 g of polyethyleneimine was dissolved in 15 ml of DI water. The solution was subsequently transferred into a Teflon-lined, stainless steel autoclave and sealed tightly. After the autoclave was heated at 150°C for 12 hours, the product was collected by centrifugation. The effect from the structure of capping agent was also investigated by adding branched PEI (M_w 10,000) and linear PEI (M_w 25,000) separately, of which products denote as CD_i and CD_b respectively.

Synthesis of benzyl bromide-carbon dots

0.2 g of freeze-dried as-synthesized carbon dots were dispersed in 0.5 ml of deionized water, 0.5 ml of benzyl bromide and 1 ml of acetone was then added. The mixture was stirred at room temperature for 24 hours. The product was washed with hexane and vacuum dried at 45°C for 12 hours.

Quantum Yield

Absolute quantum yield of carbon dots was measured with UV-3150 spectrometer and spectrofluorophotometer with 0.10 M quinine sulfate (QS) H₂SO₄ solution as the reference¹. Carbon dots solutions were prepared in five different concentrations with absorbance between 0.01 and 0.1 to minimise any re-absorption effects, because inner filter effects may cause non-linearity of absorbance in higher concentration². Then measure photoluminescence and absorbance of both samples and reference. The quantum yield of the carbon dots was calculated with the following equation.

$$\Phi_s = \Phi_r \left(\frac{m_s}{m_r} \right) \left(\frac{n_s^2}{n_r^2} \right)$$

where the subscripts s and r denote carbon dots sample and reference respectively, Φ represents the fluorescence quantum yield ($\Phi=0.54$ for QS), m is the gradient by plotting integrated fluorescence intensity against absorbance, while η represents the refractive index of the solvent used. In this case, the refractive index of QS in 0.10 M H₂SO₄ and carbon dots in deionized water are both $\eta=1.33$ ³.

TEM Sample preparation

Original concentration carbon dots solution applied on a freshly glow discharged carbon TEM grid for 3 mins. The grid was then washed twice with DI water, and then stained with uranyl acetate (UA) stain (0.5 %) for 1.5 mins to enhance the contrast of the CDs. The contrast and recognizability of the carbon dots can be enhanced by applying a band pass image filter to filter out features <5 pixels and >50 pixels.

Instrumentation

Energy-filtered TEM (EF-TEM) was conducted using Zeiss Omega 912 at 120 kV accelerating voltage. False-colored elemental mapping was measured with appropriate in-column energy filter at corresponding energy-losses, and modeled using the three-window technique. UV-Vis absorption spectrum was measured with Shimadzu UV-3150 spectrometer. Photoluminescence (PL) emission spectrum was obtained with Shimadzu RF-5301PC spectrofluorophotometer. ZetaPlus Brookhaven Instruments was used to measure size distribution of the carbon dots with dynamic light scattering. Energy-filtered TEM (EF-TEM) was conducted using Zeiss Omega 912 at 120 kV accelerating voltage. False-coloured elemental mapping was measured with appropriate in-column energy filter at corresponding energy-losses, and modelled using the three-window technique. ¹H NMR and ¹³C NMR spectra were obtained with D₂O as solvent using a Bruker 400 Ultrashield NMR spectrometer at 400MHz and 100MHz respectively.

Broth Microdilution MIC Testing

The antibacterial property of the antimicrobial carbon dots was confirmed by broth microdilution MIC testing. MIC determines the lowest concentration of the antimicrobial material that is able to inhibit the growth of the bacteria to be less than or equal to 1% after 24 hours. A gram-positive bacterium *Staphylococcus aureus* (S. aureus) and a gram-negative bacterium *Escherichia coli* (E. coli) were used for the Broth Microdilution minimum inhibitory concentration (MIC) testing. Serial carbon dots dilution was prepared in the Mueller-Hinton broth media at the following concentrations: 256 μ g/ml, 128 μ g/ml, 64 μ g/ml, 32 μ g/ml, 16 μ g/ml, 8 μ g/ml, 4 μ g/ml and 2 μ g/ml. The bacteria colonies were prepared in deionized water to match that of a 0.5 McFarland standard. Then prepare serial concentration of bacterial (128 μ g/ml, 6 μ g/ml, 32 μ g/ml, 16 μ g/ml, 8 μ g/ml, 4 μ g/ml, 2 μ g/ml and

1 µg/ml) by adding 30 ml of media to 200 µl of bacteria solution. Duplicate 100 µl each of the above solutions in a 96-well microplate to carry out for the MIC test by using a spectrophotometer. The concentration of the bacteria in the media was estimated by the absorbance at 600 nm. A sample of the bacteria with media was used as positive control of absorbance. Samples of carbon dots in media and media only were used to deduct the background of the absorbance. The growth of the bacteria can be evaluated as following equation.

$$\text{Growth (\%)} = \frac{\text{Abs}(\text{sample}) - \text{Abs}(\text{carbon dots})}{\text{Abs}(\text{bacteria in media}) - \text{Abs}(\text{media})} \times 100\%$$

In vitro gene transfection assay

The in vitro transfection assay was performed using plasmid pRL-CMV as the reporter gene in HEK293T cells. Luciferase assay is used to test the transfection efficiency of plasmid pRL-CMV that encodes Renilla luciferase. Firstly, cells were seeded into 24-well plate at a density of 5×10^4 cells/well in 0.5 mL of complete DMEM medium and incubate for 24 h. The carbon dots/pDNA complexes were prepared at weight ratios of 0.75, 1.5, 3, 4.5, 7.5 and 15. At the time of transfection, the medium in each well was replaced with 0.5 mL of serum-free DMEM medium. The complexes containing 1.0 µg of pDNA were added to each well. After 4 h of incubation, the medium was replaced with 0.5 mL of fresh complete medium and incubate for additional 20 h under standard incubation conditions. Sequentially, the luciferase gene expression was quantified using Renilla Luciferase Assay System kit (Promega, Cergy Pontoise, France). The cells were washed with PBS once and lysed with 100 µL of cell lysis buffer for 30 min with gentle rocking at room temperature. 100 µL of assay reagent (substrate and buffer) was added in 20 µL of lysis sample in 96-well white solid plate and the luciferase activity was measured on a luminometer (Infinite M200, Tecan) for 10 s of integration time. The relative light units (RLUs) were normalized against protein concentration in the cell samples, which was measured using a bicinchoninic acid assay kit (Biorad Lab, Hercules, CA). The results were collected in triplicated and expressed as relative light units per milligram of cell protein lysate (RLU/mg protein).

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3. (a) Hu, Q.; Paau, M. C.; Zhang, Y.; Gong, X.; Zhang, L.; Lu, D.; Liu, Y.; Liu, Q.; Yao, J.; Choi, M. M. F., Green synthesis of fluorescent nitrogen/sulfur-doped carbon dots and investigation of their properties by HPLC coupled with mass spectrometry. *RSC Advances* **2014**, *4* (35), 18065-18073; (b) Chandra, S.; Patra, P.; Pathan, S. H.; Roy, S.; Mitra, S.; Layek, A.; Bhar, R.; Pramanik, P.; Goswami, A., Luminescent S-doped carbon dots: an emergent architecture for multimodal applications. *Journal of Materials Chemistry B* **2013**, *1* (18), 2375-2382.