SCIENTIFIC REPORTS

OPEN

Simple Approach to Synthesize Amino-Functionalized Carbon Dots by Carbonization of Chitosan

Xin Liu, Jinhui Pang, Feng Xu & Xueming Zhang

Received: 12 May 2016

Accepted: 13 July 2016

Published: 05 August 2016

Carbon dots (CDs) as a new series of fluorescent nanomaterials have drawn great attention in recent years owing to their unique properties. In this paper, a simple carbonization approach to synthesize amino-functionalized CDs was developed by using chitosan as the carbon precursor. The as-prepared CDs possessed desirable amino function group on their surface and exhibited bright luminescence with absolute quantum yield (QY) of 4.34%, excitation-, pH-dependent and up-conversion fluorescence behaviors. Furthermore, we have investigated the cytotoxicity and biocompatibility of the as-prepared CDs, which demonstrated that the as-prepared CDs have the potential applications in biosensing, cellular imaging and drug delivery.

Carbon dots (CDs) as a new member of carbon nanomaterials have drawn a great deal of attention, owing to their chemical stability, excellent water solubility, tuneable fluorescence properties, low cost, low toxicity, good biocompatibility and environmental friendliness^{1–4}. These outstanding merits make them show promising prospects as benign candidates for various potential applications, such as fluorescent markers, bioimaging agents, photocatalysts, sensors and optoelectronic devices^{5–9}. Therefore, exploring alternative routes and starting materials for the synthesis of CDs are highly needed.

Up to now, a variety of methods have been developed to synthesize CDs, which can be roughly classified into “top-down” and “bottom-up” approaches¹⁰. The “top-down” approach includes chemical oxidation, electrochemical synthesis, arc-discharge and laser-ablation, where the CDs are formed from larger carbon materials¹¹. The “bottom-up” approach refers to obtaining CDs from molecular precursors. Generally, “bottom-up” approach consists of microwave/ultrasonic preparation, plasma treatment, hydrothermal/acidic oxidation route and carbonizing organics route^{12–14}. Nevertheless, many of these synthesis methods involve expensive or toxic starting materials, high temperature, long reaction time and further surface-passivation^{15,16}.

In recent years, the preparation of fluorescent CDs without using organic chemicals in a simple, economical and environmentally friendly way is of great interest, which is so-called the green chemistry concept¹⁷. Some green synthetic routes have been developed for the preparation of CDs by using inexpensive and renewable resources as starting materials, like watermelon peels, orange juice, chicken eggs, gelatin, etc^{11,18,19}. Remarkably, chitosan is the *N*-deacetylated derivative of chitin (a naturally abundant mucopolysaccharide), containing high amount of amino ($-NH_2$) and hydroxyl ($-OH$) functional groups, also accepted as carbon source to synthesize CDs owing to its natural, nontoxic and biocompatible properties^{20,21}.

In this work, we presented a very simple approach to synthesize amino-functionalized CDs by one-step carbonization treatment of chitosan (Fig. 1), which was scarcely studied before. In this method, neither a strong acid solvent nor surface passivation reagent was used. Both the formation and the functionalization of CDs were accomplished simultaneously. The as-prepared amino-functionalized CDs exhibited excellent fluorescence together with excitation-, pH-dependent and up-conversion fluorescence behaviors. Besides, due to their low toxicity and fine aqueous dispersibility, the as-prepared CDs showed great potential in biosensing, cellular imaging and drug delivery applications.

Results and Discussion

Physical structure of as-prepared CDs. The transmission electron microscopy (TEM) and atomic-force microscopy (AFM) images (Fig. 2) illustrated that the as-prepared CDs were quasi-spherical and monodisperse with size distribution between 1–6 nm in diameter. Besides, the high resolution transmission electron microscopy (HRTEM) image (inset of Fig. 2(a)) clearly showed the lattice spacing of 0.18 nm, which might be assigned

Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, P. R. China. Correspondence and requests for materials should be addressed to X.Z. (xm_zhang@bjfu.edu.cn)

Figure 1. Schematic illustration of the preparation of CDs from chitosan.

Figure 2. TEM (a) and AFM (b) images of as-prepared CDs.

Figure 3. (a) XRD patterns of chitosan and as-prepared CDs and (b) Raman spectrum of as-prepared CDs.

to the (102) facet of graphitic carbon¹⁵. The X-ray diffraction (XRD) pattern of chitosan and as-prepared CDs were displayed in Fig. 3(a). The spectrum of chitosan showed distinct diffraction peaks at 11.24 and 19.96°, corresponding to the amorphous peak and crystalline peak, separately²². After carbonization, the crystallinity of chitosan diminished and the spectrum of as-prepared CDs showed a broad peak at $2\theta = 22.48^\circ$, similar to the graphite lattice spacing, which was attributed to highly disordered carbon atoms^{23,24}. Additionally, the Raman spectrum of as-prepared CDs (Fig. 3(b)) revealed that although the fluorescence background signal was very strong, a relatively strong D band at 1378 cm^{-1} and G band at 1580 cm^{-1} could be obviously observed, and the ratio of intensities of I_D/I_G was calculated to be 1.15, indicating the presence of more defects or amorphous carbon as compared with sp^2 hybridization in as-prepared CDs^{25–27}, which coincided with the HRTEM and XRD results.

Chemical structure of as-prepared CDs. The Fourier transform infrared (FT-IR) spectra of chitosan and as-prepared CDs were presented in Fig. 4(a). The basic characteristic peaks of chitosan appeared at 3438 cm^{-1} (O-H and N-H stretching vibrations), 2920 and 2875 cm^{-1} (C-H stretching vibration), 1643 and 1602 cm^{-1} (N-H bending vibration), 1493 – 1245 cm^{-1} (C=C, C=N and C=C-O groups) and 1165 – 992 cm^{-1} (C-H bending vibrations of the pyranose ring)¹⁴. However, for the as-prepared CDs, the adsorption of O-H and N-H stretching vibration at 3401 cm^{-1} were decreased. Meanwhile, the C-H bending vibrations associated with pyranose ring in chitosan were completely disappeared. These differences between chitosan and as-prepared CDs might be

Figure 4. (a) FT-IR spectra of chitosan and as-prepared CDs, (b) CO_2 -TPD profile, (c) Whole survey XPS spectrum, (d) C 1s XPS spectrum, (e) O 1s XPS spectrum and (f) N 1s XPS spectrum of as-prepared CDs.

ascribed to the decomposition of the chitosan chain and pyranose ring during the carbonization process. Notably, the bands at 2930 and 1615 cm^{-1} corresponded to N-H bending vibration of amino group increased²⁸. The temperature programmed desorption of CO_2 (CO_2 -TPD) profile of as-prepared CDs (Fig. 4(b)) suggested a distinct desorption of CO_2 centered around 76°C , similar to previous reports for amino-modified solids²⁹, which might be due to the interaction between amino-functionalized CDs and CO_2 via a chemisorption mechanism. The general formula of interaction between the functional amine and the CO_2 molecules results in the formation of ammonium carbamates under anhydrous conditions can be seen in Equation (1)^{30,31}:

Also, the zeta-potential of as-prepared CDs was positive when the pH was below 6.67, indicating the existence of amine groups on the surface of as-prepared CDs together with the FT-IR and CO_2 -TPD results³².

X-ray photoelectron spectroscopy (XPS) measurement was carried out to further investigate functional groups presented on the surface of as-prepared CDs. The whole XPS survey spectrum of the as-prepared CDs (Fig. 4(c)) exhibited three obvious peaks at 284.81 , 399.33 , and 532.3 eV , which were attributed to C 1s, N 1s and O 1s, respectively. The C 1s XPS spectrum (Fig. 4(d)) can be further deconvoluted into four carbon states at 284.82 , 285.40 , 286.12 and 288.79 eV , which were attributed to C-C/C=C, C-N, C-O and C=N/C=O respectively

Figure 5. UV-Vis absorption, PL excitation and PL emission spectra of as-prepared CDs solution. Inset are the photos of as-prepared CDs solution under sunlight (left) and 365 nm UV lamp (right) illumination.

and might result in a series of emissive traps between π and π^* states of $C=C^{15}$. The O 1s spectrum (Fig. 4(e)) showed two peaks at 532.01 and 533.17 eV, which were assigned to the $C=O$ and $C-O$ bands, respectively^{28,33}. Furthermore, the N 1s spectrum (Fig. 4(f)) revealed an apparent peak at 399.83 eV, which was associated with N-H band¹⁴. The results from XPS spectra showed that the presence of hydrophilic functional groups on the surface of the as-prepared CDs without further modification. And these groups made as-prepared CDs aqueous dispersible and made a significant contribution to their optical properties³⁴.

Optical properties of as-prepared CDs. Although the fluorescence emission mechanisms remain debatable, two classes of fluorescence emission mechanisms have been proposed. The first class mechanism is related to the bandgap transitions caused by conjugated π -domains, while the second class mechanism arises from the existence of multiple surface defects³⁵. The Ultraviolet-visible (UV-Vis) absorption and photoluminescence (PL) spectra of as-prepared CDs were investigated as shown in Fig. 5. From UV-Vis absorption, it was obviously that a strong UV-Vis absorption peak located at 261 nm, which typically assigned to the π - π^* transition of aromatic sp^2 domains from the carbon core^{14,36}. As shown in the PL spectra, when excited at the maximum excitation wavelength of 310 nm, the as-prepared CDs exhibited a strong PL peak at 390 nm. The aqueous solution of as-prepared CDs was nearly transparent under visible light, while it displayed strong blue fluorescence under UV light (365 nm) excitation. (inset, Fig. 5)

Furthermore, the phenomenon of excitation dependent was clearly observed in Fig. 6(a). The emission spectra were sensitive to the excitation wavelength and the emission peak varied from 370 nm to 490 nm as the excitation wavelength increased from 250 nm to 430 nm. When the excitation wavelength increased from 250 to 310 nm, the emission peak become stronger and redshift. When excited from 310 to 430 nm, the emission peak kept redshift with remarkably decreased intensities. The excitation-dependent and redshift optical properties were similar to previous studies^{37–39}. As shown in Fig. 6(b), the up-conversion fluorescence emission phenomenon was observed that when as-prepared CDs were excited by longer wavelength (450–550 nm), shorter wavelength (400 nm) was emitted, which may be caused by the two or multiphoton active process⁴⁰. Figure 6(c) illustrated that the optical responses of the as-prepared CDs with pH values ranging from 1 to 13. It was clearly that PL emission wavelength of as-prepared CDs were stable in the pH range of 5 to 9. In strong acidic condition, the emission wavelength evolved to the longer wavelength, while moved to shorter wavelength in strong basic solution, implying that the as-prepared CDs were very sensitive to acid/base solvent. These tunable fluorescence emission properties of as-prepared CDs were particular attractive for sensing, *in vivo* bioimaging and synthesizing novel photocatalysts^{7,11,40}.

Fluorescence decay curve and the exponential fitting curve of as-prepared CDs were showed in Fig. 6(d). The fitting formula is:

$$R(t) = B_1 e^{(-t/\tau_1)} + B_2 e^{(-t/\tau_2)} + B_3 e^{(-t/\tau_3)} + B_4 e^{(-t/\tau_4)} \quad (2)$$

where t is time, B_1, B_2, B_3 and B_4 are fractional intensities, τ_1, τ_2, τ_3 and τ_4 are fluorescence lifetime. The parameters generated from iterative reconvolution of the decay with the instrument response function (IRF) were listed in the inset of Fig. 6(d). And according to formula:

$$\tau_{ave} = \frac{B_1 \tau_1 + B_2 \tau_2 + B_3 \tau_3 + B_4 \tau_4}{B_1 + B_2 + B_3 + B_4} \quad (3)$$

we calculated the amplitude-weighted average fluorescence lifetime (τ_{ave}) of as-prepared CDs to be 6.0921 ns, which suggested that the as-prepared CDs are suitable for optoelectronic and biological application^{17,41}. Additionally, the value of absolute quantum yield (QY) was measured to be 4.34%, which was comparable to the reported carbon dots^{2,42,43}.

Cytotoxicity and cells imaging. CCK-8 assay was used to evaluate the cytotoxicity of the CDs to living cells. As shown in Fig. 7, the cell viability were estimated to be greater than 80% after 24 h of incubation upon

Figure 6. (a) Excitation-dependent PL emission spectra and (b) Up-conversion PL emission spectra of as-prepared CDs. (c) PL spectra of as-prepared CDs dispersed in different pH solutions and (d) Time-resolved PL decay and fitting curves for as-prepared CDs.

Figure 7. Cell viability of LLC cells in the presence of different concentration of as-prepared CDs.

addition of the as-prepared CDs over concentration ranged from 0 to 200 $\mu\text{g/mL}$, which confirmed the low toxicity, excellent biocompatibility and safety *in vitro* and *in vivo* applications of the as-prepared CDs^{6,44}. Furthermore, in order to investigate the imaging capability, we induced the as-prepared CDs into onion root tip cells. The confocal laser scanning microscopy (CLSM) images of onion root tip cells labeled with the as-prepared CDs for 12 h at 25 °C were shown in Fig. 8. Obviously, the as-prepared CDs have penetrated into the cells and labeled both the cell nuclei and membrane and shown significant green fluorescence emission under the 405 nm wavelength excitation. This result indicated that the as-prepared CDs possessed good biocompatibility and could serve in bioimaging and drug delivery applications^{45,46}.

Conclusions

In summary, the amino-functionalized CDs have been successfully prepared by a simple carbonization approach. This green, cheap and convenient process represented a potential advance for large-scale production. The as-prepared CDs possessed desirable amino function group on the surface and exhibited bright luminescence

Figure 8. CLSM images of onion root tip cells labeled with as-prepared CDs at 25 °C for 12 h (a) Bright field, (b) Fluorescent image excited with a 405 nm laser and (c) Overlap of the corresponding bright field image and the fluorescence image.

with absolute quantum yield (QY) of 4.34%, excitation-, pH-dependent and up-conversion fluorescence behaviors. Furthermore, the as-prepared CDs showed good stability, less toxicity, biocompatibility and excellent fluorescence imaging ability. In the future, the as-prepared CDs could be utilized promisingly in optoelectronic materials, photocatalysts, cellular imaging and drug delivery applications.

Methods

Materials. All chemicals, including chitosan (Sinopharm Chemical Reagent Co., Ltd. China), sulfuric acid (H_2SO_4 ; Beijing Chemical Works, China), sodium hydroxide (NaOH; Beijing Chemical Works, China) and phosphate buffer saline (PBS; Leagene, China), were in analytical grade and used without further purification. Deionized water was used throughout the whole experiment.

Preparation of CDs. CDs were prepared via carbonization of chitosan at 300 °C for 2 h at a heating rate of 5 °C/min under a nitrogen atmosphere. After cooled down to room temperature, the dark brown products were mechanically ground to fine powders. After that, obtained powders were dispersed in water, and the CDs were collected by filtrating for further characterization and use.

Characterization. Transmission electron microscopy (TEM) and high resolution transmission electron microscopy (HRTEM) micrographs were recorded on JEM-2100F (JEOL, Japan) operating at 200 kV. Atomic-force microscopy (AFM) investigation was done by a scanning probe microscope (BRUKER MULTIMADE 8, Bruker, USA). The crystal structure of samples was analyzed by an X-ray diffractometer (SHIMADZU XRD-6000, Shimadzu corporation, Japan) with Cu radiation ($\lambda = 0.154 \text{ nm}$) at 40 kV and 40 mA. The Raman spectrum was recorded on Perkin Elmer Spectrum GX (Perkin Elmer, UK) at room temperature. Fourier-transform infrared (FT-IR) spectroscopy data were acquired using a Perkin Elmer Spotlight 400 imaging system (Perkin Elmer, UK). The X-ray photoelectron spectroscopy (XPS) measurements were performed on Thermo Scientific Escalab 250Xi XPS system (Thermo Fisher Scientific Ltd., UK) using Al K α source. The temperature programmed desorption of CO_2 (CO_2 -TPD) measurement was carried out on an AutoChem II 2920 analyser (Micrometrics, USA) fitted with a thermal conductivity detector (TCD). Ultraviolet-visible (UV-Vis) absorption spectra were carried out with UV2310II spectrometer (Techcomp, China). The fluorescence spectra were measured with a fluorescence spectrometer (F-7000, Hitachi High Tech CO., Japan). Fluorescence decay curves and photoluminescence (PL) absolute quantum yield (QY) for as-prepared CDs were measured at room temperature using an Edinburgh FLS 980 spectrometer (Edinburgh Instruments, UK) equipped with an integrating sphere under 310 nm excitation from a 450 W xenon lamp. The absolute fluorescence quantum yield was calculated as follows:

$$\eta = \frac{E_B - E_A}{S_A - S_B} \quad (4)$$

where η is the absolute fluorescence quantum yield, the indices “A” and “B” referred to the reference sample (deionized water) and carbon dots aqueous solution, separately, and S_A , S_B , E_A and E_B referred to the integral of the scans. The specific calculation was handled by using the Edinburgh FLS 980 software package (Quantum Yield Wizard)^{47,48}.

Effect of pH on the PL of as-prepared CDs. The as-prepared CDs aqueous solutions were adjusted to the various target pH values by adding diluted H_2SO_4 or NaOH solution, then the corresponding fluorescence spectra were measured upon excitation at 310 nm separately.

Cytotoxicity evaluation. The cytotoxicity of as-prepared CDs was evaluated by a CCK-8 assay⁴⁴. Lewis Lung Carcinoma (LLC) cells were seeded at a density of 4×10^3 cells per well in 180 mL culture medium and incubated for 24 h. Then, the cells were treated with different concentrations of as-prepared CDs solution at 37 °C in a humidified incubator with 5% CO_2 for 24 h. Then, 20 mL of CCK-8 solution was added to each well and incubated

for 1 h at 37 °C. The absorbance at 450 nm was measured using an infinite M200 microplate spectrophotometer (Tecan, Switzerland). The cell viability was expressed as percentage of absorbance relative to control, and the control was obtained in the absence of as-prepared CDs.

Cell imaging. Onion root tip cells were incubated with as-prepared CDs solution for 12 h at 25 °C, then washed with PBS for several times. The cellular localization was visualized using a confocal laser scanning microscope (SP-5, Leica, Germany) with laser excitation of 405 nm^{49,50}.

References

1. Zhu, S. *et al.* Highly photoluminescent carbon dots for multicolor patterning, sensors, and bioimaging. *Angew Chem Int Ed Engl.* **52**, 3953–3957 (2013).
2. Wang, C., Sun, D., Zhuo, K., Zhang, H. & Wang, J. Simple and green synthesis of nitrogen-, sulfur-, and phosphorus-co-doped carbon dots with tunable luminescence properties and sensing application. *RSC Adv.* **4**, 54060–54065 (2014).
3. Sun, Y. P. *et al.* Host-Guest Carbon Dots for Enhanced Optical Properties and Beyond. *Sci Rep.* **5**, 12354 (2015).
4. Liu, Y., Zhao, Y. & Zhang, Y. One-step green synthesized fluorescent carbon nanodots from bamboo leaves for copper(II) ion detection. *Sensors Actua B-Chem.* **196**, 647–652 (2014).
5. Jamieson, T. *et al.* Biological applications of quantum dots. *Biomaterials.* **28**, 4717–4732 (2007).
6. Luo, P. G. *et al.* Carbon “quantum” dots for optical bioimaging. *J. Mater. Chem. B.* **1**, 2116–2127 (2013).
7. Li, H., Kang, Z., Liu, Y. & Lee, S.-T. Carbon nanodots: synthesis, properties and applications. *J. Mater. Chem.* **22**, 24230–24253 (2012).
8. Cao, L. *et al.* Carbon dots for multiphoton bioimaging. *J Am Chem Soc.* **129**, 11318–11319 (2007).
9. Araújo, T. C. *et al.* Hybrid heterostructures based on hematite and highly hydrophilic carbon dots with photocatalytic activity. *Appl Catal B-Environ.* **182**, 204–212 (2016).
10. Wang, Y. & Hu, A. Carbon quantum dots: synthesis, properties and applications. *J. Mater. Chem. C.* **2**, 6921–6939 (2014).
11. Lim, S. Y., Shen, W. & Gao, Z. Carbon quantum dots and their applications. *Chem Soc Rev.* **44**, 362–381 (2015).
12. Liu, S.-S. *et al.* Hair-derived carbon dots toward versatile multidimensional fluorescent materials. *J. Mater. Chem. C.* **2**, 6477–6483 (2014).
13. Guo, X., Wang, C. F., Yu, Z. Y., Chen, L. & Chen, S. Facile access to versatile fluorescent carbon dots toward light-emitting diodes. *Chem. Commun.* **48**, 2692–2694 (2012).
14. Liu, Y. *et al.* One-step synthesis of robust nitrogen-doped carbon dots: acid-evoked fluorescence enhancement and their application in Fe³⁺ + detection. *J. Mater. Chem. A* **3**, 17747–17754 (2015).
15. Sahu, S., Behera, B., Maiti, T. K. & Mohapatra, S. Simple one-step synthesis of highly luminescent carbon dots from orange juice: application as excellent bio-imaging agents. *Chem Commun (Camb).* **48**, 8835–8837 (2012).
16. Yang, Y. *et al.* One-step synthesis of amino-functionalized fluorescent carbon nanoparticles by hydrothermal carbonization of chitosan. *Chem Commun (Camb).* **48**, 380–382 (2012).
17. Xue, M., Zou, M., Zhao, J., Zhan, Z. & Zhao, S. Green preparation of fluorescent carbon dots from lychee seeds and their application for the selective detection of methylene blue and imaging in living cells. *J. Mater. Chem. B.* **3**, 6783–6789 (2015).
18. Zhu, L., Yin, Y., Wang, C.-F. & Chen, S. Plant leaf-derived fluorescent carbon dots for sensing, patterning and coding. *J. Mater. Chem. C.* **1**, 4925–4932 (2013).
19. Song, Y., Zhu, S. & Yang, B. Bioimaging based on fluorescent carbon dots. *RSC Advances.* **4**, 27184–27200 (2014).
20. Rinaudo, M. Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* **31**, 603–632 (2006).
21. Kumar, M. N. V. R. A review of chitin and chitosan applications. *React. Funct. Polym.* **46**, 1–27 (2000).
22. Zhang, Y., Xue, C., Xue, Y., Gao, R. & Zhang, X. Determination of the degree of deacetylation of chitin and chitosan by X-ray powder diffraction. *Carbohydr Res.* **340**, 1914–1917 (2005).
23. Wang, F., Kreiter, M., He, B., Pang, S. & Liu, C. Y. Synthesis of direct white-light emitting carbogenic quantum dots. *Chem Commun (Camb).* **46**, 3309–3311 (2010).
24. Wang, W., Li, Y., Cheng, L., Cao, Z. & Liu, W. Water-soluble and phosphorus-containing carbon dots with strong green fluorescence for cell labeling. *J. Mater. Chem. B.* **2**, 46–48 (2014).
25. Qu, S., Wang, X., Lu, Q., Liu, X. & Wang, L. A biocompatible fluorescent ink based on water-soluble luminescent carbon nanodots. *Angew Chem Int Ed Engl.* **51**, 12215–12218 (2012).
26. Machado, C. E. *et al.* Influence of Inert and Oxidizing Atmospheres on the Physical and Optical Properties of Luminescent Carbon Dots Prepared through Pyrolysis of a Model Molecule. *Chem. Eur. J.* **22**, 4556–4563 (2016).
27. Amama, P. B., Cola, B. A., Sands, T. D., Xu, X. & Fisher, T. S. Dendrimer-assisted controlled growth of carbon nanotubes for enhanced thermal interface conductance. *Nanotechnology.* **18**, 385303 (2007).
28. Liu, S. *et al.* Hydrothermal treatment of grass: a low-cost, green route to nitrogen-doped, carbon-rich, photoluminescent polymer nanodots as an effective fluorescent sensing platform for label-free detection of Cu(II) ions. *Adv Mater.* **24**, 2037–2041 (2012).
29. Madden, D. & Curtin, T. Carbon dioxide capture with amino-functionalised zeolite-β: A temperature programmed desorption study under dry and humid conditions. *Microporous Mesoporous Mater.* **228**, 310–317 (2016).
30. Knowles, G. P., Graham, J. V., Delaney, S. W. & Chaffee, A. L. Aminopropyl-functionalized mesoporous silicas as CO₂ adsorbents. *Fuel Process Technol.* **86**, 1435–1448 (2005).
31. Amornvadee Veawab, P. T. & Amit Chakma. Corrosion Behavior of Carbon Steel in the CO₂ Absorption Process Using Aqueous Amine Solutions. *Ind. Eng. Chem. Res.* **38**, 3917–3924 (1999).
32. Xiao, D., Yuan, D., He, H. & Lu, J. Microwave-assisted one-step green synthesis of amino-functionalized fluorescent carbon nitride dots from chitosan. *Luminescence.* **28**, 612–615 (2013).
33. Wu, Q., Li, W., Tan, J., Wu, Y. & Liu, S. Hydrothermal carbonization of carboxymethylcellulose: One-pot preparation of conductive carbon microspheres and water-soluble fluorescent carbon nanodots. *Chem. Eng. J.* **266**, 112–120 (2015).
34. Li, X., Rui, M., Song, J., Shen, Z. & Zeng, H. Carbon and Graphene Quantum Dots for Optoelectronic and Energy Devices: A Review. *Adv. Funct. Mater.* **25**, 4929–4947 (2015).
35. Luo, P. G. *et al.* Carbon-based quantum dots for fluorescence imaging of cells and tissues. *RSC Adv.* **4**, 10791–10807 (2014).
36. Wang, C. *et al.* A hydrothermal route to water-stable luminescent carbon dots as nanosensors for pH and temperature. *Carbon.* **82**, 87–95 (2015).
37. Hola, K. *et al.* Photoluminescence effects of graphitic core size and surface functional groups in carbon dots: COO[−] induced red-shift emission. *Carbon.* **70**, 279–286 (2014).
38. Pan, D. *et al.* Observation of pH-, solvent-, spin-, and excitation-dependent blue photoluminescence from carbon nanoparticles. *Chem Commun (Camb).* **46**, 3681–3683 (2010).
39. Wang, F. *et al.* Graphene quantum dots as a fluorescent sensing platform for highly efficient detection of copper(II) ions. *Sensors Actua B-Chem.* **190**, 516–522 (2014).
40. Zhu, S. *et al.* Surface Chemistry Routes to Modulate the Photoluminescence of Graphene Quantum Dots: From Fluorescence Mechanism to Up-Conversion Bioimaging Applications. *Adv. Funct. Mater.* **22**, 4732–4740 (2012).
41. Peng, J. *et al.* Graphene quantum dots derived from carbon fibers. *Nano Lett.* **12**, 844–849 (2012).

42. Liu, H., Ye, T. & Mao, C. Fluorescent carbon nanoparticles derived from candle soot. *Angew Chem Int Ed Engl.* **46**, 6473–6475 (2007).
43. Ling, Y. *et al.* Bright Light-Emitting Diodes Based on Organometal Halide Perovskite Nanoplatelets. *Adv Mater.* **28**, 305–311 (2016).
44. Zhang, X. *et al.* Carbon-dots derived from nanodiamond: photoluminescence tunable nanoparticles for cell imaging. *J. Colloid Interface Sci.* **397**, 39–44 (2013).
45. Ray Chowdhuri, A., Tripathy, S., Haldar, C., Roy, S. & Sahu, S. K. Single step synthesis of carbon dot embedded chitosan nanoparticles for cell imaging and hydrophobic drug delivery. *J. Mater. Chem. B.* **3**, 9122–9131 (2015).
46. Huang, C.-L. *et al.* Application of paramagnetic graphene quantum dots as a platform for simultaneous dual-modality bioimaging and tumor-targeted drug delivery. *J. Mater. Chem. B.* **3**, 651–664 (2015).
47. Tang, M., Huang, Y., Wang, Y. & Fu, L. An ytterbium complex with unique luminescence properties: detecting the temperature based on a luminescence spectrum without the interference of oxygen. *Dalton Trans.* **44**, 7449–7457 (2015).
48. Dorneanu, P. P. *et al.* Solvent effects on the photophysical properties of poly[1,4-dihydroxyanthraquinoneimine-1,3-bis(phenylene-ester-methylene)tetramethyldisiloxane]. *Spectrochim. Acta, Part A.* **134**, 218–224 (2015).
49. Chen, B. *et al.* Fluorescent probe for highly selective and sensitive detection of hydrogen sulfide in living cells and cardiac tissues. *Analyst.* **138**, 946–951 (2013).
50. Li, F. *et al.* Mg/N double doping strategy to fabricate extremely high luminescent carbon dots for bioimaging. *RSC Adv.* **4**, 3201–3205 (2014).

Acknowledgements

We are grateful for the financial support of this research from the Fundamental Research Funds for the Central Universities (No. 2016JX03), Natural Science Foundation of China (No. 31470606), China National Funds for Distinguished Young Scientists (31225005) and China Ministry of Education (No. 111).

Author Contributions

X.Z. and X.L. conceived the project and designed the experiments; X.L. wrote the main manuscript text; X.L. and J.P. performed the experiments and analysed the data; X.Z. and F.X. supervised and directed the project; All authors reviewed the manuscript.

Additional Information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Liu, X. *et al.* Simple Approach to Synthesize Amino-Functionalized Carbon Dots by Carbonization of Chitosan. *Sci. Rep.* **6**, 31100; doi: 10.1038/srep31100 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© The Author(s) 2016