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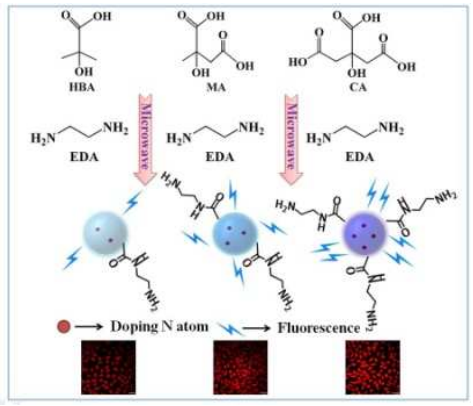

Carbon source with different number of carboxyl group greatly affects the photoluminescence and quantum yield of the carbon nanodots.

Surface passivated carbon nanodots prepared by microwave assisted pyrolysis: effect of carboxyl group in precursors on fluorescence property

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Abstract

Carbon nanodots (CDs) have become one of the hottest topic in the fluorescent nanoparticles family. During the preparation of CDs, two necessary materials are involved, namely, carbon source and passivator. Both themgreatly affects the photoluminescence (PL) and quantum yield (QY) of the CDs. We hypothesized that the interaction of carbon sources with passivation agents would make a great difference in the PL properties of CDs. 2-hydroxyisobutyric acid (HBA), malic acid (MA) and citric acid (CA) with similar chemical structure and different number of carboxyl groups for dehydration and carbonization were selected as the carbon source to investigate the effect of carboxyl groups on the fabrication of PL properties of CDs. The three kinds of CDs were synthesized by one-step microwave-assisted pyrolysis in the presence of 1,2-ethylenediamine as a passiviator. The maximum QY of the as-prepared CDs fabricated from HBA, MA and CAwere 11.81%, 24.52% and 56.42%, respectively. In addition, the fluorescent lifetime and nitrogen content increased with the number of carboxyl groups in carbon source molecule. The different number of carboxyl group was shown to influence the PL of CDs due to the different ability to conjugate animo groups.Moreover, the results of cell co-culture indicated that the resultant CDs were highly biocompatible and bright, holding great potential for biomedical applications.

Keywords:carbon nanodots, microwave-assisted pyrolysis, carboxyl group, surface passivation

1. Introduction

Photoluminescent carbon nanodots (CDs), since their accidental discovery during the electrophoretic purification of single-walled carbon nanotubes derived from arc-discharge soot,[1] have attracted intensive attentionin recent years due to their unique physical and chemical properties and great potential in biological labeling, bioimaging, biosensing, drug and gene delivery as well as optoelectronic device applications.[1-6] It is well known that CDs show similar photophysical performance and photochemical stability to that of the already commercialized fluorescent semiconductor quantum dots (QDs). However, QDs are usually composed of heavy metals as the essential elements to achieve high performance fluorescence which tend to cause toxicity in biomedical applications.[4-8] It is encouraging that CDs can overcome this drawback due to the absence of those toxic elements, which results in a non-toxic response.

In the past few years, many methods have been developed to prepare CDs, including laser ablation, arc-discharge, electrochemical oxidation, acid dehydration, microwave heating, combustion/thermal and supported routes, etc. [3,4,9] These methods can be classified into two main groups: top-down and bottom-up methods. [4,5] The top-down methods were primarily based on the post treatment of nanocarbon exfoliated from various larger carbon structures, such as carbon nanotubes, graphene, nanodiamond and commercial activated carbon.[3,4,10-12] The bottom-up approaches generally involved the carbonization of suitable precursors, such as glucose, sucrose, glycerol, citric acid, orange juice, candle soot or natural-gas burner, carbohydrates, chitosan gel, bovine serum albumin, bombyxmori silk and dextrin.[13-23] By these methods, two types of CDs were prepared, namely, the “Naked” CDs and the surface passivated CDs. “Naked” CDs without surface functionalization were shown to exhibit colorful fluorescence emissions but with generally low to very low quantum yield (QY) (lower than 10% or even 1%),[24,25] but the passivation of the CDs with polymers or other organic molecules can increase the QYs by several folds.[6,16,26-28]

During the preparation of passivated CDs, two important ingredients areinvolved, namely, carbon source and passivator. Both carbon source and passivator may have greatly affected

the PL and QY of the CDs obtained. In the previous research, we have fabricated the CDs with the same carbon source and different amine-based passivators by one-step microwave. The result demonstrated that amine molecules of the passivator not only acted as surface passivation agents, but also as N-doping precursors by forming amide bonds during the fabrication of CDs, and the dual functions were shown to considerably enhanced the fluorescence.[29]

On the other hand, the carbonization was a necessary process for synthesizing CDs, and complete carbonization was beneficial for achieving the best optical performance.[5] This process was closely related with the kind of the chosen carbon sources due to the different reactivity or reacting routes in carbonization and interaction with passivation or doping agents. Quite different PL properties were observed for CDs prepared by various carbon sources ranging from candle soot and saccharides to natural bioresources. [17,30-33] For example, as reported by our previous studies, the CDs obtained from citric acid and acrylic acid with the same passivation agent ethylenediamine (EDA) showed different optical properties. [29,34] Up to now, however, there has been no systematical study to illuminate the effect of the interaction of different carbon sources with passivation agents on the PL properties of CDs, which we believe will be beneficial to achieve better PL quality.

In this study, in order to investigate the effect of carboxyl groups on the fabrication of luminescent CDs,2-hydroxyisobutyric acid (HBA), malic acid (MA) and citric acid (CA) (Fig.1) were selected as the carbon sources for below reasons: (1) they possess similar chemical structures; (2) all of them contain one hydroxyl group in a molecule; (3) they all contain carboxyl groups which can facilitate the dehydration and carbonization, but the number is different. EDA was used as the surface passivation agent. The water soluble CDs were synthesized by one-step microwave-assisted pyrolysis.The PL properties of the resultant CDs were characterized and the influence of carboxyl group on the PL and QY of CDs were discussed. Furthermore, the potential use of the CDs for biomedical imaging was demonstrated by laser scanning confocal microscopy imaging of L929 cells.

2. Materials and methods

2.1. Materials

2-Hydroxyisobutyric acid (HBA, 99%) and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide (MTT, 98%) were obtained from Alfa Aesar. Citric acid (CA, 99%), malic acid (MA, 99%) and 1,2-ethylenediamine(EDA), were supplied by GuangFu Technology Development CO, LTD, China. Quinine sulfate (98%, suitable for fluorescence) was purchased from Fluka. All other reagents were of analytical grade and used without further purification.

2.2. Synthesis of CDs

The CDs were synthesized by a facile green route of microwave assisted pyrolysis method. The details of the preparation of HBA-CDs are given below as a typical example. 1.04 g (0.01 mol) HBA was dissolved in 10.4 ml ultrapure water to give a final concentration of 0.1 g/ml, and then different amount of EDA from 0.0025 mol to 0.04 mol was added to the solution under vigorous stirring. Subsequently, the clear transparent solution was put into a domestic microwave oven (700 W) and heated for different time. The product was dissolved and dialyzed against pure water through a dialysis membrane (MWCO=500) for 3 days after cooled down to room temperature. Finally, a clear, light yellow-brown aqueous solution containing HBA-CDs was lyophilized to collect dry HBA-CDs. The different HBA-CDs were synthesized by varying the molar ratios of carboxyl groups of HBA to amino groups of EDA. The MA-CDs and CA-CDs were prepared by similar method.

2.3. Instrumentation and characterizations

UV-Vis absorption was conducted on a TU-1810 UV-Vis Spectrophotometer (Pgeneral, China). Photoluminescence (PL) emission was characterized using FLS920 fluorometer (Edinburgh Instruments, Britain). The normalized spectra were obtained by dividing the intensity of each PL spectrum by the maximum value of its own. The morphology and microstructure of the CDs were measured by high-resolution transmission electron

microscopy (HRTEM) on a Philips Tecnai G2 F20 microscope (Philips, Netherlands) with an accelerating voltage of 200 kV. The samples for HRTEM were made by dropping a droplet of aqueous solution onto a 300-mesh copper grid coated with a lacy carbon film. Fourier transform infrared spectroscopy (FT-IR) spectra of the samples were measured on a Nicolet 380 spectrometer (Thermo, America). The compositions of CDs were confirmed by elemental analysis with Vanio-EL (Elementar Analysensysteme GmbH, Germany). The X-ray photoelectron spectroscopy (XPS) spectra of the samples were measured on a Kratos AXIS Ultra DLD X-ray Photoelectron Spectroscopy (Shimadzu, Japan).

2.4. Measurement of QY

The QY of the CDs were determined by a comparative method. Quinine sulfate (literature QY: 54%) which was dissolved in 0.1 M H_2SO_4 was used as a standard sample to calculate the QY of test samples. The test samples were dissolved in ultra pure water at different concentrations. All the absorbance values of the solutions at the excitation wavelength were measured with UV-Vis spectrophotometer. Photoluminescence (PL) emission spectra of the sample solutions were recorded by FLS920 fluorometer at the maximum excitation wavelength. For HBA-CDs, MA-CDs and CA-CDs, the excitation wavelength were 340 nm, 380nm and 360 nm, respectively. The integrated fluorescence intensity is the area under the PL curve in the wavelength range from 385 to 700 nm. Then, a graph was plotted using the integrated fluorescence intensity against the absorbance and a trend line was added for each curve with intercept at zero. Absolute values were calculated according to the following equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where the subscripts ST and X denote standard and test, respectively, Φ is the fluorescence QY, Grad is the gradient from the plot of integrated fluorescence intensity *vs* absorbance, and η is the refractive index of the solvent. In order to minimize the re-absorption effects, absorbance in the 10 mm fluorescence cuvette should never exceed 0.1 at the excitation

wavelength.

2.5. Cell culture, cytotoxicity assay and confocal microscopy

L929 (murine aneuploid fibrosarcoma cell line) was purchased from Peking Union Medical College (Beijing, China). The cells were cultured in RPMIi-1640 Medium (1640, HyClone), containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin at 37 ºC in 5% CO_2 humidified atmosphere.

The cytotoxicity of CDs was evaluated by MTT assay using L929 cells. Aliquots of cell suspension in l640 medium supplemented with 10% FBS containing a density of 4×10^4 cells/well L929 cells were seeded into a 96-well plate and incubated overnight at 37^{o}C, in 5% CO_2 humidified atmosphere. Then, the culture medium was removed and the CDs at the increasing concentrations from 0.5 to 10 mg/ml were added into each well. After incubating for 24 h, the medium was removed and replaced with 200 μl fresh complete medium containing 20 μl MTT (5 mg/ml in PBS). The plate was further incubated for 4 h. Finally, all medium was removed and 150 μl/well DMSO was added, followed by shaking for 30 min at 37^{o}C. The absorbance of each well was measured at 570 nm using a Synergy HT Multi-Mode Microplate Reader (BioTek, USA) with pure DMSO as a blank. Non-treated cells were used as a control and the relative cell viability (mean% ± SD, n = 3) was calculated as follows:

$$\text{Cell viability (\%)} = Abs_{sample}/Abs_{control}\times100\%$$

Where Abs_{sample} and $Abs_{control}$ denote the absorbance of each well with CDs and without CDs, respectively.

For confocal microscopy, L929 cells were seeded on a coverslip in 6-well plate 12 h before use. Then, the culture medium was replaced by 2.5 ml fresh medium containing 2 mg/ml CDs and the cells were incubated for another 24 h. Then the cells were washed with isotonic PBS (pH=7.4) three times, and fixed with 4% paraformaldehyde solution in PBS at 4ºC overnight. The samples were examined under a Leica confocal laser scanning microscope (Mannheim, Germany) equipped with a UV laser (351/364 nm), an Ar laser (457/488/514nm) and a HeNe

laser (543/633 nm).

3. Results and discussion

3.1. Influential factors of CDs

In order to investigate the effect of carboxyl groups on the fabrication of luminescent CDs by the microwave assisted pyrolysis method, HBA, MA and CAwere employed as carbon sources and EDA as a surface passivation agent to prepare CDs. The three kinds of sources have similar structure and all contain carboxyl groups and hydroxyl groups which could facilitate the dehydration and carbonization processes, while the difference is the number of carboxyl groups (Fig. 1).

Firstly, the influence of different pyrolysis conditions (e.g., the molar ratios of carboxyl/amine groups and the microwave irradiation time) on PL properties of the CDs was investigated to gain the optimum conditions for each system. For HBA-CDs as shown in Fig. 2, the optimum microwave times were 3 min, 4 min, 2 min, 2 min and 4 min when the molar ratios of carboxyl groups to amino groups are 1:0.5, 1:1, 1:2, 1:4 and 1:8. For the MA-CDs (Fig. S1), the best microwave times were 4 min, 2 min, 2 min, 3 min and 3 min corresponding to the above ratio of carboxyl groups/amino groups. As shown in Fig. S2, it can be seen that the optimum microwave times of CA-CDs were 2 min for each carboxyl/amino group ratio. For each reaction system, the PL emission reduced when the treatment time was either longer or shorter than the optimum time. Usually, the synthesis of CDs can be achieved through three stages: dehydration and nanoparticle formation, surface passivation of nanoparticle and growth of CDs. [8] It was believed that a short microwave irradiation time was not enough to achieve complete surface passivation for the best optical performance. However, an excess of water was evaporated when the irradiation time was longer; as a result, the CD particles were over-heated and their surface structure was destroyed, and thus the PL emission decreased. [26,29]

At the same time, the effect of the molar ratio of carboxyl groups to amino groups on PL intensity was investigated. In this experiment, the different molar ratios of carboxyl groups to

amino groups were controlled by adding equal amount of carbon source (1 g) and deionized water (10 mL) with different amount of EDA. Each system was heated in microwave oven (700 W) for 2 min. As shown in Fig. 3, the PL intensity firstly increased and then peaked at the molar ratio of carboxyl groups to amino groups of 1:2 and then decreased with the increment of EDA. It indicated that the ratio 1:2 was the optimum ratio among others. It can be explained that adding proper amount of EDA into the reaction system played a role of surface passivation, which was beneficial to improving the PL intensity. [2,26,35] During the microwave pyrolysis, it was found that the mixture in microwave oven was easy to be over heated when the content of EDA exceeded a certain amount (data not shown). We speculated that part of the free EDA volatilized with water vapour during the microwave pyrolysis process, and the excessive amount of EDA led to more water loss. As a result, the reaction system tended to be over heated. Thus the surface structure of CDs was destroyed, leading to the PL emission decrease.

3.2. Fluorescent property of CDs

To further explore the optical properties of the as-prepared CDs, a detailed study with different excitation wavelengths ranging from 300 to 460 nm was carried out (Fig. 4). The PL peak of HBA-CDs kept steady only under the excitation wavelength of 300-340 nm (Fig. 4A). It was also observed that the PL peak of MA-CDs (Fig. 4B) remained at 450 nm for the excitation wavelength of 340-400 nm, but the PL peak shifted for about 50 nm when the excitation wavelength was larger than 400 nm. The CA-CDs exhibited an excitation-dependent PL behavior only when the excitation wavelength was larger than 400 nm, and the peak remained almost unchanged (at 450 nm) for the excitation wavelength between 300-400 nm (Fig. 4C), which was similar as our previous data.[29] It is reported that the PL of CDs was attributed to the presence of surface energy traps which became emissive upon surface passivation [26] and different energy levels were associated with different "surface states" which were affected by different surface functionalization.[5,25,26,36,37] The different relationships between excitation wavelength and emission spectrum over 300-460 nm indicated that the surface defect of the three kinds of CDs were different. Since the same

passivant was used for the three CDs, the passivating extent was probably varied with carbon source, leading to the different surface defects. We further supposed that the result may be related to the carboxyl group which can conjugate with different amount of amino groups of passivant EDA. This will be verified in the next experiment.

For the fluorescent CDs, QY is a significant parameter for their application. Quinine sulfate (QY 54%) was selected as a standard sample to calculate the QY of CDs. The increasing amount of EDA enhanced the QY to a certain extent, and then the QY appeared a decreasing tendency when the molar ratios of carboxyl groups to amino groups were more than 1:2 (Fig. S3, Table 1), which was in accordance with the PL emission spectra (Fig. 3). This phenomenon was in agreement with the reported work.[29,35] An explanation is that the existence of EDA enhanced the PL emission to a certain degree, but excessive EDA resulted in over-heated CDs with excessive amount of water loss and the destruction of CDs surface. The maximum QY of the as-prepared CDs fabricated from HBA, MA and CA were 11.81%, 24.52% and 56.42%, respectively (Fig.S3). The QY increased with the number of carboxyl group in carbon source molecule. We speculate that the QY of CDs depended on the ability of carbon source to capture amino groups. Although the conditions of all reaction systems were equal, including the water volume, molar ratios of amino groups to carboxyl groups and reaction time, the carboxyl groups' capability of conjugating with amino groups was varied for different carbon sources. There are three carboxyl groups, two carboxyl groups and one carboxyl group in HBA, MA and CA, respectively. Under the same conditions, there were more opportunities to conjugating amino groups for molecules with more carboxyl groups, which endowed the CDs with more amino groups on their surfaces. In the following experiments, the CDs prepared with molar ratio of carboxyl to amine groups at 1:2 were characterized.

As shown in Fig. S4, although there was no fluorescence under visiblelight, a bright blue color PL was observed upon excitation of the aqueous solutions of the three kinds of CDs at 365 nm. It was obvious that the fluorescence intensity of the three followed the sequence of CA-CDs > MA-CDs > HBA-CD, which was in agreement with the PL emission analysis and

the different QY. It was demonstrated previously that the carboxylate groups formed on the particle surface were of great significance to the PL of CDs.[9,25] In our case, the sequence of the fluorescence intensity of the three kinds CDs corresponds to the degree of the interaction between COOH and NH_2. When more NH_2 moieties were captured by COOH in carbon source, the better passivation effect resulted in higher fluorescence intensity.

3.3. Characterization of CDs

Fig. 5 showed HRTEM images and the size distribution of the three kinds of CDs. The average particle diameters of HBA-CDs, MA-CDs and CA-CDs were 1.5 nm, 1.4 nm and 1.4 nm, respectively. There was no significant difference in particle size among HBA-CDs, MA-CDs and CA-CDs, and all of them possessed a narrow size distribution of 1-2 nm in diameter. As reported in the literature, most CDs were observed to be amorphous, without any well-resolved lattice fringes. [33,35] The absence of discernible lattice structures suggested that all of the as-prepared CDs were in the form of amorphous carbon.

As shown in Fig. S5, the FT-IR spectra of the HBA-CDs, MA-CDs and CA-CDs samples were similar with those reported by other literature.[8,29,35,38] All of them showed absorption peaks at near 1645 cm^{-1}, 1530 cm^{-1} and 1400 cm^{-1}, which can be attributed to the stretching vibrations of C=O, N-H and C-N, respectively, indicating the existence of amide bond on the surface of the CDs.

The fluorescence lifetimes of the three CDs were obtained via transient fluorescence spectra measurement (Fig. S6). There were two lifetimes for all of the CDs and the average lifetime increased with the increment of carboxyl group in the carbon source molecules. The average lifetime of HBA-CDs was 8.1 ns which contained two lifetime components of 13.2 ns (~44.9%) and 4.0 ns (~55.1%) (340 nm excitation, decay time at 400 nm emission); the average value for MA-CDs was 9.4 ns, consisting of two components of 14.85 ns (~38.5%) and 6.02 ns (~61.5%) (380 nm excitation, decay time at 460 nm emission); CA-CDs exhibited an average lifetime of 14.55 ns, also two components were inclusive, 16.2 ns (~78.6%) and 8.5 ns (~21.4%) (360 nm excitation, decay time at 450 nm emission). Fluorescence lifetime

was closely related to the surface states of the CDs. We speculated these surface states were greatly influenced by surface passivation, which inevitably depended on the interaction between the carbon sources and the passivant.

In order to verify our hypothesis, the elemental analysis and XPS were applied to characterize the CDs. The elemental analysis data were shown in Table 1. It was obvious that the N content increased in the order of HBA-CDs, MA-CDs and CA-CDs, indicating that more N elements were incorporated in the CDs derivatived from carbon sources with more COOH groups. As shown in the above discussion, the QY and fluorescent lifetime were also in the same order. The high-resolution N 1s spectra of all the CDs (Fig. 6) showed strong signals from both amide-N and doping N atoms according to our previous work reported,[29] indicating the presence of both type N elements, which was confirmed in the C 1s spectra as shown in Fig. S7. During the microwave-assisted pyrolysis, the formation of CDs and the surface passivation processes were accomplished simultaneously. Usually, amino-group enriched molecules could improve the PL properties of the resultant CDs by acting as N-doping agent and the passivation agent. For example, the TTDDA and diamine-terminated oligomeric poly(ethylene glycol) which contained primary amine groups were used as passivation agents to form amide bonds on the surface of pre-formed CDs for the fluorescence enhancement. [10,26,39-41] As a N-containing carbon source, the ethylenediaminetetraacetic acid could enhance the PL properties of the resultant CDs by doping N atoms into the carbon core. [38,42] Certainly, for N-containing molecules, the formation of surface amide groups and the N-doping can co-exist during the preparation of CDs and both contribute to the improvement of the PL properties.

3.4. Cytotoxicity and cell imaging of CDs

To exlpore the potential applications of the resultant CDs in biomedical imaging, L929 cells were used to evaluate the cytotoxicity of the as-prepared CDs by the MTT assay. As shown in Fig. S8, L929 cells retained viability of about 95% even at a concentration of 3 mg/mL and 80% at 6 mg/mL for HBA-CDs, MA-CDs and CA-CDs. It demonstrated that all

these three kinds of CDs possessed extremely low cytotoxicity, as reported elsewhere. [8,29,40]

Based on the evaluation of the biocompatibility, the CDs were introduced into L929 cells for in vitro bioimaging and the images were recorded using a confocal microscopy. Compared with the control cells in the absence of CDs (Fig. 7a), L929 cells incubated with CDs became brighter owing to the strong fluorescence emitting from CDs in cytosol, showing blue, green, orange and red colors upon excitation at 405 nm, 488 nm, 561 nm and 640 nm, respectively (Fig. 7 b-d). It indicated that large amount of CDs had been internalized into cells. These CDs were of remarkably high biocompatible and ideal for bioimaging. The PL stability of the three kinds of CDs to pH was investigated. As shown in Fig. S9, all of them were stable at pH 4-10. The PL decreased when pH was higher than 10 or lower than 4. The stability decreased in the order: HBA-CDs, MA-CDs and CA-CDs. The relative QY changed reversibly in response to pH variation, which was similar as reported. [35] This phenomenon can be assigned to the ionization of carboxyl groups on the CDs, resulting in the more extent ionization with more carboxyl groups which directly influenced the stability of CDs. Anyway, the pH range of 4-10 over which the stability of the fluorescence could be kept was wide enough for bioimaging.

4. Conclusion

In summary, this study is the first attempt to examine the effect of carboxyl group in carbon source on the fluorescence property of CDs prepared by microwave mediated pyrolysis method. The highly luminescent CDs from HBA, MA and CA sources with EDA as passiviation agent were prepared. The maximum QY of the as-prepared CDs fabricated from HBA, MA and CAwere 11.81%, 24.52% and 56.42%, respectively. The carboxyl groups had a great effect on the CDs design and optimization. The QY, fluorescent lifetime and N content increased with the number of carboxyl groups in the carbon source molecule, due to their enhanced ability to conjugate with amino groups. Moreover, our preliminary results indicated that the resultant CDs were highly biocompatible and held great potential for biomedical applications.

Acknowledgements

The authors gratefully acknowledge the support for this work from National Science and Technology Major Project of China (Grants 2012ZX10004801-003-007, 2012AA022603).

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Table 1 The results of elemental analysis and QY of CDs

Samples	C%	H%	N%	O (Calculated, %)	QY (%)
HBA-CDs	44.75	9.78	12.13	33.34	11.81
MA-CDs	38.69	5.08	14.95	41.28	24.52
CA-CDs	38.27	5.28	22.52	33.93	56.42

Captions:

Fig. 1. Synthesis of HBA-CDs, MA-CDs and CA-CDs in the present of EDA.

Fig. 2. PL spectra (excited at 340 nm) of HBA-CDs prepared with different molar ratios of carboxyl groups to amino groups: (A) 1:0.5, (B) 1:1, (C) 1:2, (D) 1:4, (E) 1:8 at different microwave pyrolysis time periods. Each sample has the same absorbance value.

Fig. 3. PL emission spectra of HBA-CDs (A: excited at 340 nm), MA-CDs (B: excited at 380 nm) and CA-CDs (C: excited at 360 nm) prepared with different ratio of amino groups to carboxyl groups at the same time period.

Fig. 4. PL emission spectra of the three kinds of CDs and the normalized PL emission spectra (insets) at different excitation wavelength: (A) HBA-CDs, (B) MA-CDs and (C) CA-CDs.

Fig. 5. HRTEM images of HBA-CDs (A), MA-CDs (B) and CA-CDs (C), scale bar: 20 nm.

Fig. 6.XPS spectra of HBA-CDs (A), MA-CDs (B) and CA-CDs (C); XPS N 1s spectra of HBA-CDs (D), MA-CDs (E) and CA-CDs (F).

Fig. 7. Laser scanning confocal microscopy images of L929 cells without labeling as a negative control (a) and HBA-CDs (b), MA-CD (c), CA-CDs (d) labeled L929 cells, scale bar: 40.00 μm.

Fig. 1

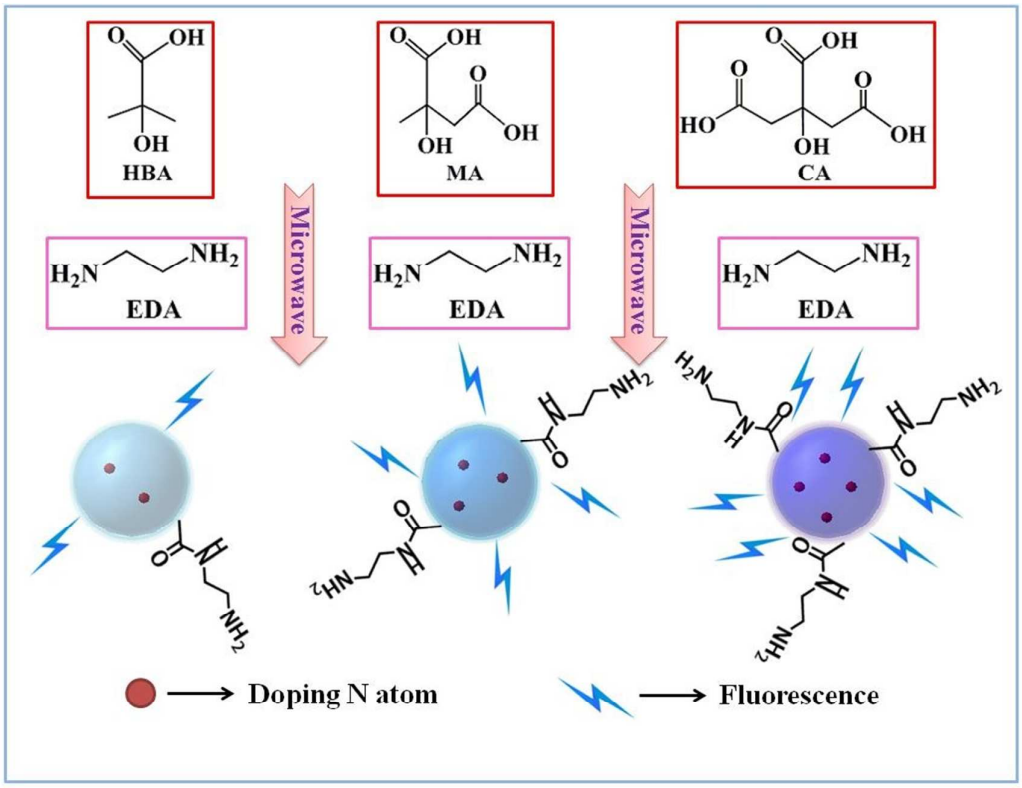

Fig. 2

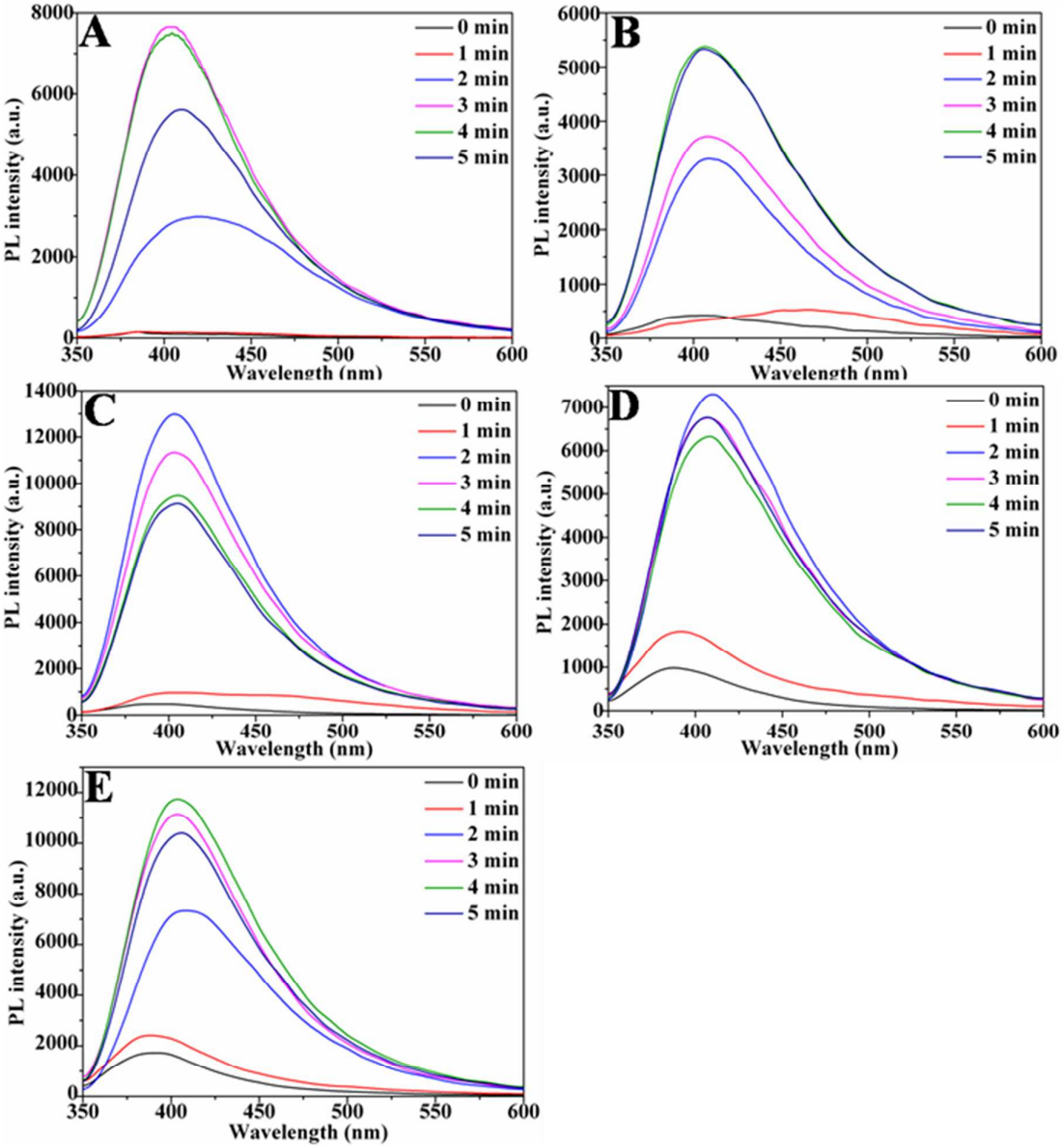

Fig. 3

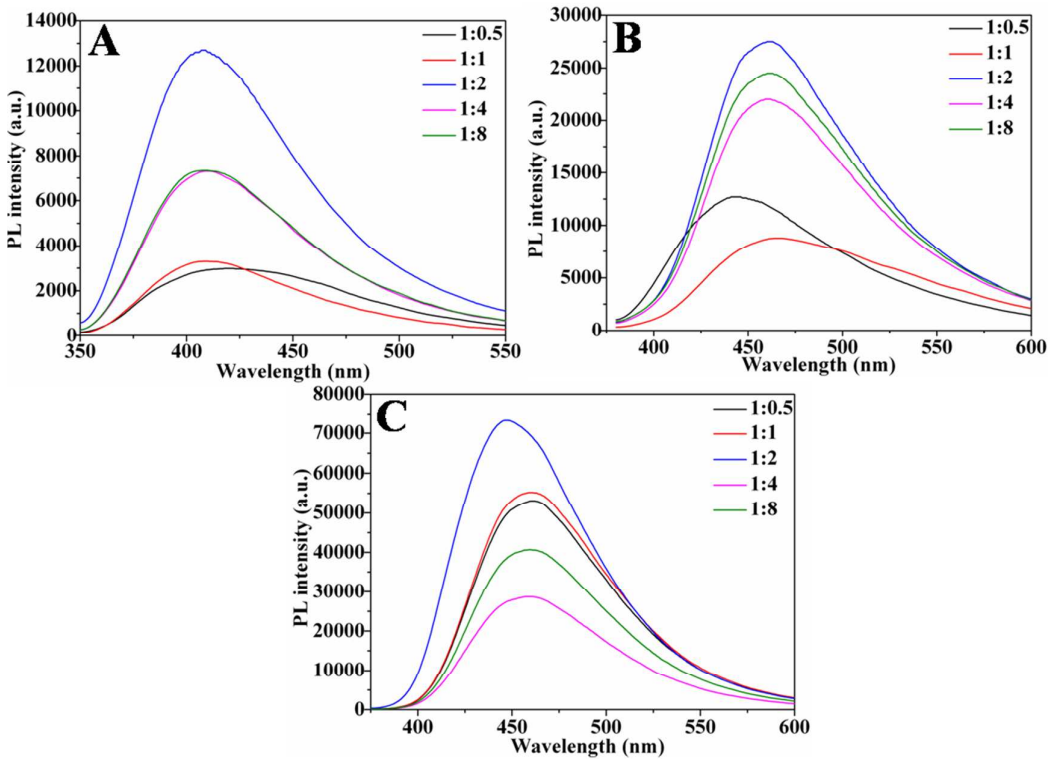

Fig. 4

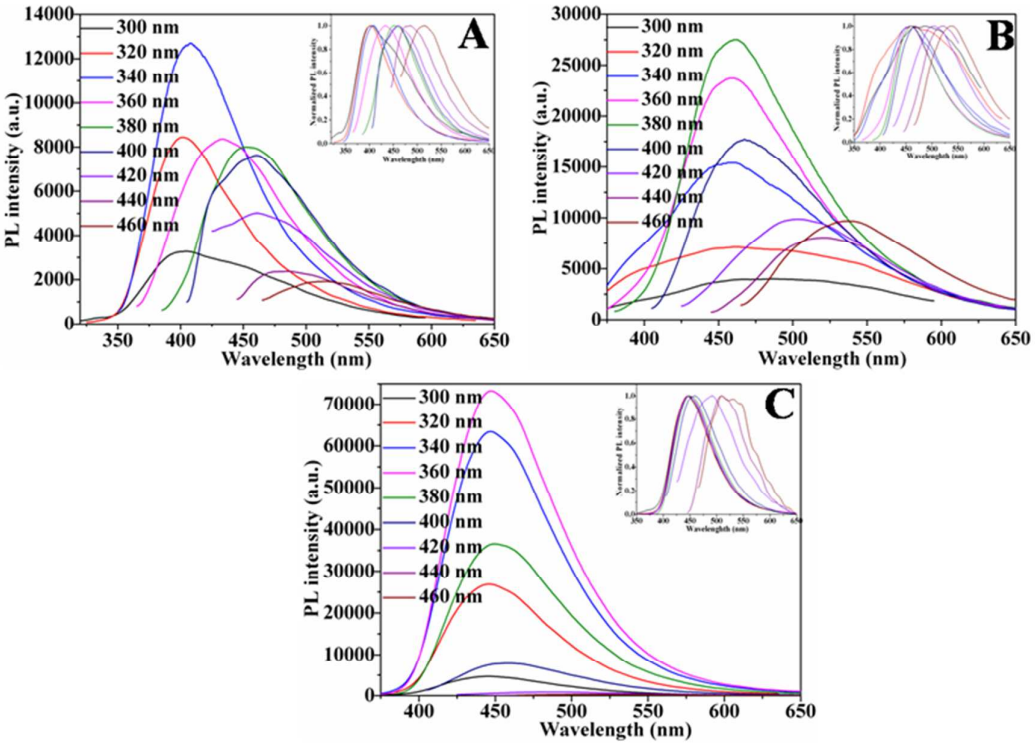

Fig. 5

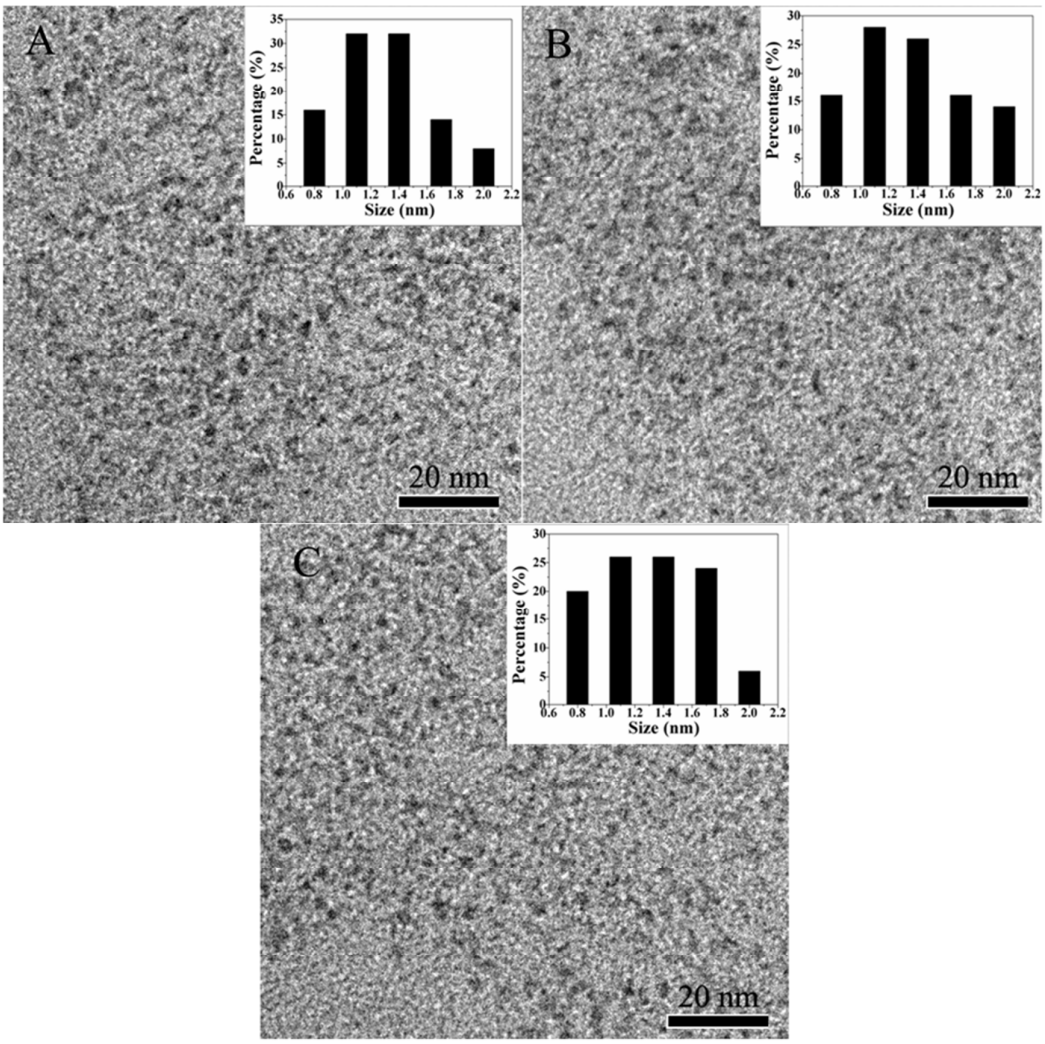

Fig. 6

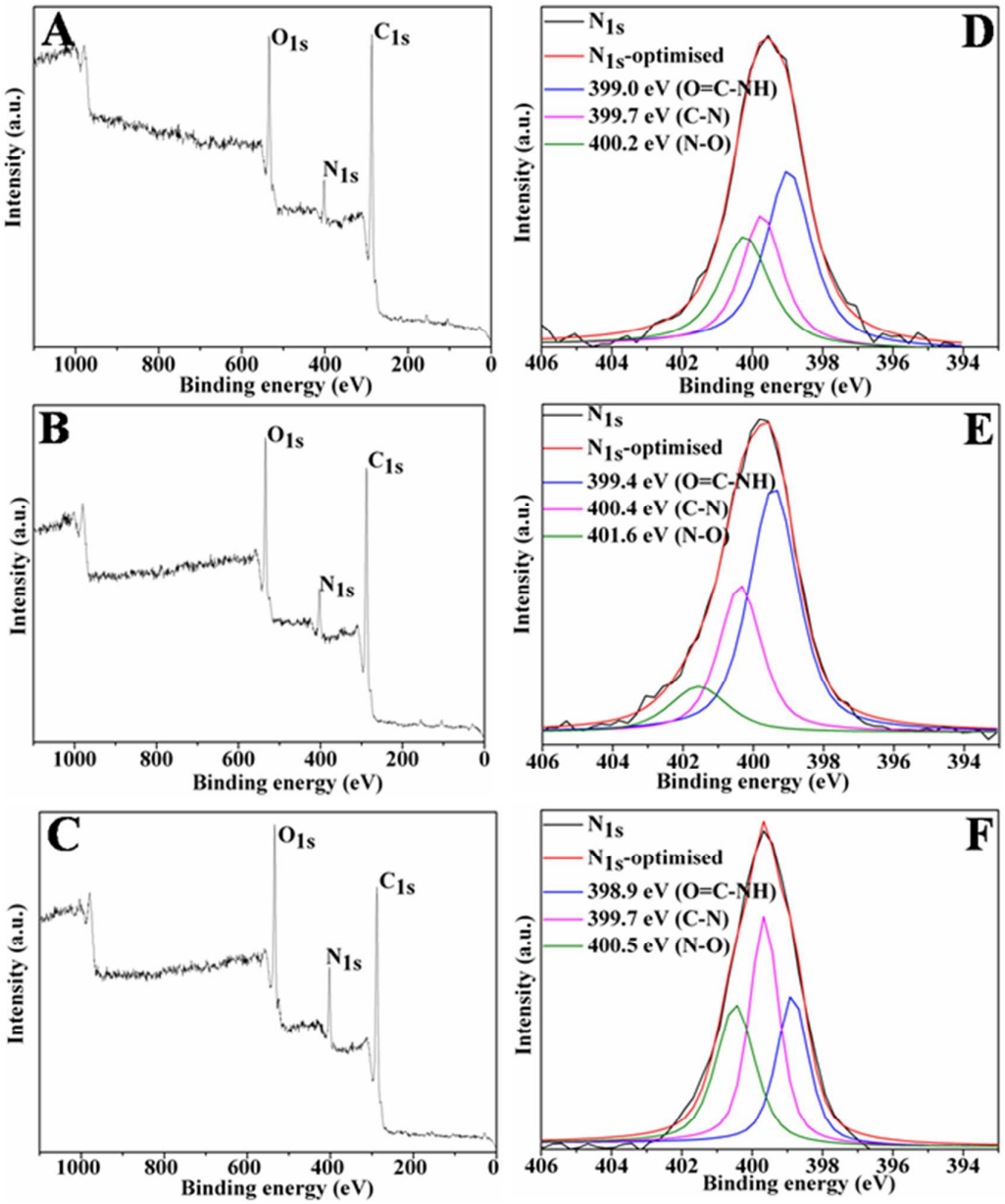

Fig. 7