Fluorescent property of carbon dots extracted from cigarette smoke and the application in bio-imaging

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Abstract: Cigarette smoke is one of the six major pollution sources in the room air. It contains large number of particles with size less than 10 nm. There exist carbon dots (CDs) in cigarette smoke which have strong fluorescence and with good bio-compatibility and low toxicity. CDs in cigarette smoke can be applied in bio-imaging which has great potential applications in the integration of cancer diagnosis and treatment. In this paper, CDs were extracted from cigarette smoke. Then, sodium borohydride was added to CDs aqueous solution for reduction and the reduced CDs (R-CDs) were used for biological cell imaging. The results indicate that the CDs with the particle size <10 nm in cigarette smoke are self-assembled by the polymerized polycyclic aromatic hydrocarbons (PAHs) and ammonium nitrite which are disk nano-structure composed of \(sp^2\)/\(sp^3\) carbon and oxygen/nitrogen groups or polymers. Sodium borohydride can reduce the carbonyl group on the surface of CDs to hydroxyl group and increase the ratio of the Na 1s ratio of the CDs from 1.86 to 7.42. The CDs can emit blue fluorescence under ultraviolet irradiation. After reduction, the R-CDS have the intensity of fluorescence 7.2 times than before and the fluorescence quantum yield increase from 6.13% to 8.86%. The photoluminescence (PL) wavelength of R-CDs have red-shift of 7 nm which was due to the increasing of Na element ratio. The onion epidermal cells labeled with R-CDs show that the CDs could pass through the cell wall into the cell and reach the nucleus. The cell wall and the nucleus could be clearly visualized.

CDs also shows low toxicity to human bronchial epithelial cells (BEAS-2B) with good biological activity. The obtained results indicate that the CDs and R-CDs have good fluorescent property which could be used as bio-imaging agent.

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1. Introduction

Carbon dots (CDs) are new types of zero-dimensional (0D) carbon-based nanomaterials with excellent properties such as strong fluorescence, good water solubility, low toxicity, good photostability, chemical stability, and bio-compatibility [1–4]. Therefore, it has great potential applications in light emitting devices, green lighting, biological imaging, cell labeling, photocatalysis, and many other fields [5, 6]. CDs can be prepared from abundant raw materials which can be classified as biomass and non-biomass. Biomass raw materials include plants, animals and their derivatives or wastes [7]. Non-biomass raw materials are chemical reagents, fossil fuels, and minerals such as coal and graphite [8]. However, biomass carbon sources can be easily...
gained from nature with which are diverse and renewable. Therefore, the investigations of CDs made from biomass has attracted lots of attentions.

It is worth noting that the ultra-fine particles with size \( <10 \text{ nm} \) generated by the combustion of biomass and fossil energy are very common such as cigarette smoke, soot from coal-fired power generation, smoke from forest fires, volcanic ash, and etc. These nanoscale particles cause serious pollution to the environment of human life and are harmful to human health. However, some relevant studies only focus on the impact of particles with size \( >20 \text{ nm} \) on human health [9–18] whereas the effect of CDs with size \( <10 \text{ nm} \) have not received enough attentions [19–21].

Tobacco contains more than 7000 kinds of chemical substances and about 400 of them can cause toxicity to human body with more than 50 kinds of carcinogens including arsenic, cadmium, formaldehyde, and benzo pyrene [22]. Cigarette smoke is the most common tobacco product. It’s one of the main sources of human exposure to fine particles in the air. 92% of cigarette smoke is gas which mainly contains carbon monoxide, carbon dioxide, nitrogen oxides, volatile low molecular alkanes, and alkenes, etc. Besides, the other 8% is particulate matters (PM) [27–29] which is smoke dust with particle size of 0.1–2 \( \mu \text{m} \) and an average diameter of 0.2 \( \mu \text{m} \). The smoke dust becomes tar after condensation. Each cigarette produces \( 6.3 \times 10^9 \) particles per second which are about 20–35 mg tar. It has been confirmed that they contain more than 4000 carcinogens such as nicotine, polycyclic aromatic hydroxyl, benzo pyrene, and \( \beta \)-naphthylamine [23]. Carbonyl compounds are main components of cigarette smoke. The carbonyl particles in cigarette smoke account for 11-19% of the total particulate mass and some of them are toxic and may be carcinogenic or mutagenic to human being [24].

When the cigarettes are burning, the surface temperature of the burning area is about 300 °C and the central combustion temperature is about 900 °C [25,26]. The organic matters in tobacco will produce a large number of fluorescent CDs with the particle size of 7 nm-10 \( \mu \text{m} \) [30]. The number of PM with particle size \( \leq 0.1 \mu \text{m} \) is roughly the same as that of 0.1-2.0 \( \mu \text{m} \) [31]. The PM with particle size of 0.1-2.0 \( \mu \text{m} \) contribute to the most of the total PM mass. A large number of CDs with size \( \leq 10 \text{ nm} \) are often overlooked because they are difficult to detect with small mass and size.

In order to explore the structure of particles with size \( <10 \text{ nm} \) in cigarette smoke and the role of surface carbonyl and hydroxyl groups played, CDs were extracted from the static burning smoke of 60 cigarettes made in China. The structure, chemical composition, surface groups, and fluorescence characteristics of CDs were characterized by TEM, XPS, XRD, PL, absorption spectrum, and particle size distribution. The carbonyl group in CDs was reduced by NaBH\(_4\) and converted to hydroxyl group to passivate the CDs and enhance the fluorescence. Meanwhile, the R-CDs are injected into onion epidermal cells to study the beneficial effect of R-CDs on the imaging quality of plant cells. The toxicity test is performed to evaluate the toxicity of CDs on BEAS-2B.

2. Experiment

2.1. Materials and experimental instruments

The cigarettes (Yunyan brand) were purchased from local supermarket. Ethanol and sodium borohydride are pure analytical reagents. The deionized water used in the experiment was produced by Master-S15 deionized water production machine (Shanghai Hetai Instrument Co., LTD). Cigarette smoke collection equipment includes SHZ-III recirculating water vacuum pump (Shanghai Yarong Biochemical Instrument Factory), TG16G table top centrifuge (Yancheng kaite Experimental Instrument Co., LTD.), and KUDOS ultrasonic cleaning equipment (Shenzhen Xinbao Instrument Co., LTD.). The fluorescence spectrum of CDs was measured by a fluorescence spectrometer F9818012 (Shanghai lenguang technology Co., LTD). The absorption spectra were measured by a UV-Vis spectrophotometer (Specord200, Germany). The morphology and micro-structures of the CDs were characterized by using the transmission electron microscopy.
(TEM, JEM 2100). The X-ray photoelectron spectroscopy (XPS) of CDs were measured by using PHI5000 Versa Probe II photoelectron spectrometer with Al $K_{\alpha}$. The infrared absorption spectrum was measured by a Fourier transform infrared spectrometer (Nicoletis10, USA).

2.2. Synthesis of CDs with the cigarette

Firstly, 400 ml of deionized water and absolute ethanol were injected into two elution bottles, respectively. Absolute ethanol was used as the solvent to dissolve tar in flue gas, and deionized water was used as the solution to extract CDs in flue gas. Then, 60 cigarettes (Yunyan brand) burns in turn and place them at one of the end of the vent. Cigarette smoke is pumped into the bottle through a vacuum pump. CDs are extracted with deionized water by using the good water solubility of CDs. The CDs solution was a light yellow liquid. After centrifuged and filtered by a filter membrane with a pore size of 220 nm, the CDs solution was stored in a refrigerator for later use.

In order to enhance the intensity of fluorescence of the CDs, the CDs was passivated. 10 ml of the collected CDs solution with a concentration of 0.2 mg/mL and 60 mg sodium borohydride ($\text{NaBH}_4$) are added to the CDs aqueous solution and it was stirred at room temperature for full reaction. After reaction, the CDs solution was filtered by a filter membrane with a pore size of 220 nm.

2.3. Cell imaging study and the toxicity of CDs

The inner membrane of onion epidermis was torn off with tweezers from the fresh onions. CDs was dropped into the epidermis for 5 mins with natural absorption. Then, the structure of onion epidermis cells was observed under ultraviolet, violet, and green light with a fluorescence microscope. In order to evaluate the toxicity of CDs, BEAS-2B cultured to logarithmic growth stage are implanted into 96-well culture plate with 2000 cells per well. 24 hours later, the cells are stuck to the wall. Then, suck out them from the medium and added 200 $\mu$L/well CDs solution with different concentrations (0.08-1.38 mg/mL) and the solvent is serum-free cell medium. After culturing for 24 hours, CCK-8 assay is used to measure the cell activity.

3. Results and Discussions

The particulate matter (PM) produced by cigarette combustion usually contains polycyclic aromatic hydrocarbons (PAHs), organic nitrates, ethylpyridine, and other organic compounds. PAHs are mainly produced from incomplete combustion of organic matter. They widely exist in environmental pollutants worldwide and are known for their carcinogenic, mutagenic, and teratogenic properties. Among more than 500 carcinogenic compounds, PAHs and its derivatives account for more than 200. The ratio of PAHs in PM is about 57 % while naphthalene ($\text{C}_10\text{H}_8$) is the simplest and most abundant PAHs in smoke [29]. During the burning of cigarettes, organic matter in tobacco is firstly pyrolysed to PAHs and then polymerized during the burning process to

![Fig. 1. The possible formation mechanism and structural characteristics of CDs in smoke.](image)
form a large number of CDs with size <10 nm. Fig. 1 shows the possible formation mechanism and structural characteristics of CDs in cigarette smoke. PAHs and ammonium nitrite contained in the tar of cigarette smoke are polymerized and self-assembled into disk nano-structure composed of $sp^2/sp^3$ carbon crystal nuclei and oxygen/nitrogen group or polymer. The structure diagram of CDs is shown in the right side of Fig. 1.

In Fig. 2, we show the TEM image, particle size distribution of CDs, and HRTEM image of CDs' lattice. As can be seen from Fig. 2 (a), the morphology of CDs is quasi-circular and with good dispersion. Fig. 2 (b) shows that the diameter of CDs is between 1-4 nm. The diameter particle distribution is close to normal distribution with an average diameter of 3 nm. Fig. 2

![Infrared absorption spectra of CDs and R-CDs](image)

**Fig. 3.** Infrared absorption spectra of CDs and R-CDs (after modifications with NaBH$_4$). The region of the vibrations for different bonds are indicated.
Fig. 4. The full XPS spectrum (a) and C1s peak fitting diagram (b) of CDs. The full XPS spectrum (c) and C1s peak fitting diagram (d) of R-CDs.

(c) shows that the core region of a single CD is crystalline structure with clear lattice fringes. The crystal plane spacing is about 0.21 nm, which is corresponding to the (100) crystal plane of graphene [32,33].

In order to study the changes of carbon skeleton structure and surface groups after adding NaBH₄ modifier to CDs solution, the samples were measured by Fourier infrared absorption spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). Fig. 3 shows the infrared absorption spectra of CDs solution and R-CDs solution after reduction by NaBH₄. As we can seen in Fig. 3, the absorption peaks of these two curves are approximately the same. The peaks at 3445 cm⁻¹ and 2925 cm⁻¹ belong to the stretching vibration peak of O-H and C-H bonds, respectively. Peaks at 1586 cm⁻¹, 1384 cm⁻¹, 1358 cm⁻¹, and 966 cm⁻¹ were generated by stretching vibration of C=O, C=C, C-N, and C-O bonds, respectively [34,35]. The peaks at 737 cm⁻¹ and 527 cm⁻¹ are caused by the C-H stretching vibration [36]. After NaBH₄ was added to the CDs solution, CDs is reduced to R-CDs. As can be seen from Fig. 3, the C=C bond in the carbon core region increase significantly with the enhancement of the stretching vibration of O-H bond. Meanwhile, C-O/C=O also increase significantly which indicates that the number of hydroxyl groups on the surface of R-CDs is significantly more than that of carbonyl groups. This is beneficial to the improvement of the yield and fluorescence lifetime of CDs [37,38].

Fig. 4 (a) shows the full spectrum of XPS of CDs extracted from PM dispersed in deionized water filtered from cigarette smoke. The XPS analysis of CDs shows that the CDs without the modification of NaBH₄ mainly consists of three elements, C, O, and N, with the atomic percentages of 64.5%, 27.6%, and 4.16%, respectively. It also contains small amounts of Na and Si which are due to the metal in the cigarette and the Si element in the quartz glass used as the test substrate. Fig. 4 (b) shows the C1s peak fitting diagram of CDs. The C1s peak can be divided into three peaks at 284.9 eV, 286.4 eV, and 288.3 eV which correspond to C-C/C=C,
C-O/C-N and C=O/C=N bonds, respectively [39]. Fig. 4 (c) shows the full spectrum of XPS after the reduction of CDs extracted from cigarette smoke by NaBH₄. The R-CDs after reduction are mainly composed of C and O, and their atomic percentages are C: 74.18% and O: 18.4%, respectively. Other trace elements are Na and Cl in flue gas and Si in the quartz glass. After CDs was reduced by NaBH₄, C and Na element increases and O element decreases. The content of nitrogen element in CDs becomes zero. Fig. 4 (d) is the peak fitting diagram of C1s of R-CDs. There are three peaks located at 284.9 eV, 285.9 eV, and 288.5 eV which belong to C-C, C-O and C=O bonds, respectively [40]. The results indicate that NaBH₄ can reduce the carbonyl group on the surface of the CDs to hydroxyl group and increase the Na 1s ratio of the CDs group from 1.86 to 7.42. Thus, the CDs can be passivated and it can reduce the toxicity of CDs. The XPS results are in line with the FTIR results.

Fig. 5 (a) shows the absorption spectra of CDs and R-CDs (after reduction). The CDs before reduction has an obvious absorption peak at 264 nm and a weak shoulder peak at 288 nm. The absorption peak at 264 nm is attributed to the $\pi - \pi^*$ transitions generated by C=C in aromatic hydrocarbons. The shoulder peak at 288 nm is the absorption induced by the $\pi - \pi^*$ transitions generated by C=O bond [41]. For the R-CDs, the intensity of the absorption peak is obviously weakened while the peak position remains unchanged. Fig. 5 (b) shows the value of optical band-gap of CDs which is calculated with the relationship between absorption coefficient and band-gap through $\alpha h\nu = \frac{1}{2} (h\nu - E_g)$, where $\alpha$ is absorption coefficient, $h\nu$ is photon energy, $E_g$ is band gap. The optical band-gaps of CDs and R-CDs are estimated to be 2.8 eV and 2.7 eV, respectively. The linear region of the indirect band-gap semiconductor absorption spectrum has a shoulder structure which means that the calculated band-gap parameters are for indirect band-gaps.

In Fig. 6, the solid curves show the fluorescence emission spectrum of CDs. It can be seen that the emission range is between 400-700 nm and the peak position is at 456 nm. The peak of emission spectrum does not change with the excitation wavelength. The dashed curves show the fluorescence emission spectra of R-CDs reduced by sodium borohydride in order to enhance the fluorescence intensity of CDs. The fluorescence intensity can be calculated through the integral area of the fluorescence curve. We can see that the intensity of the fluorescence emission with an excitation wavelength 360 nm is significantly enhanced with 7.2 times after reduction. This is due to the reduction of carbonyl group to hydroxyl group by NaBH₄, which reduces the non-radiative transition caused by carbonyl group and thus increases the fluorescence intensity. By the way, the optimal excitation wavelength and fluorescence emission peak are red-shifted which is due to the increasing of Na element ratio. This is analogous to a doped semiconductor, where the
impurity levels $E_D$ and $E_A$ in the forbidden band correspond to the donor and acceptor energy levels, respectively. Here, $E_D$ is equivalent to surface states, which are mainly induced by the surface groups and donor impurities of CDs or R-CDs. $E_A$ is the acceptor energy level formed by the metal ion Na$^+$ in R-CDs after reduction of NaBH$_4$. When the percentage of Na elements increase from 1.86 % in CDs to 7.42 % in R-CDs, the width of the impurity level formed by Na$^+$ increases and the energy gap between $E_D$ and $E_A$ decreases. This could result in a 7 nm red-shift of the fluorescence wavelength of R-CDs compared with CDs [42].

A combined fluorescence lifetime and steady-state fluorescence spectrometer (Edinburgh Instruments, UK) equipped with an integrating sphere was used to measure the fluorescence quantum yield and fluorescence decay curve at room temperature. Fig. 7 shows the fluorescence lifetime decay curves of CDs and R-CDs. The fluorescence decay can be fitted by a double-exponential decay model through

$$R(t) = A_0 + A_1 \exp (-t/t_1) + A_2 \exp (-t/t_2),$$

where $A_0$ is the background PL intensity and $A_1$ and $A_2$ are the fractional contributions to PL emission from two transition channels with corresponding decay time or lifetime. Through fitting, the fluorescence lifetimes of CDs and R-CDs are 6.78 ns and 9.54 ns, respectively. The Channel-1 with CDs contributes 71.41 % of the PL emission, while the Channel-2 contributes 26.07 %. The Channel-1 with R-CDs contributes 0.54 % of the PL emission, while the Channel-2 contributes 94.1 %. Specifically, Channel-1 relates to the transition between the intrinsic carbon base states in CDs, while Channel-2 corresponds to the transition with the surface states in CDs via non-radiation and radiative electronic relaxations [38]. Such a result indicates that the radiative electronic transition can be acquired in nanoseconds in CDs. By the way, the width of the impurity level formed by Na$^+$ would also attribute to the non-radiative process and the fluorescence process of R-CDs is more complicated than that of CDs [43]. Thus, the fluorescence lifetime of R-CDs is longer than that of CDs. Moreover, the fluorescence quantum yield of CDs is calculated through

$$Q = Q_S \cdot \frac{I_S}{I} \cdot \frac{A}{A_S} \cdot \frac{\eta}{\eta_S}$$

Fig. 6. Fluorescence spectra of CDs and R-CDs in cigarette smoke.
where $Q_S$ is the quantum yield of the fluorescence for a standard sample for reference. $I$ and $I_S$ are the integrated emission intensities of the CDs sample and the standard sample, respectively. $A$ and $A_S$ are respectively the absorbance of the prepared sample and standard sample at the same excitation wavelength. $\eta$ and $\eta_S$ are respectively the refractivity of the prepared sample and standard sample. In this study, the fluorescence quantum yield of CDs is calculated relatively to the standard fluorescent substance quinine sulfate. The instrument used in this experiment has been calibrated with quinine sulfate standard solution during system setup. Thus, the fluorescence quantum yield of CDs can be measured by inserting the sample into the integrating sphere. The quantum yield of CDs and R-CDs are 6.13% and 8.86%, respectively.

In Fig. 8, we show micrograph of onion epidermal cells with blue fluorescence under (a) ultraviolet light (330-400 nm), (b) green fluorescence under violet light (395-415 nm), and (c) red fluorescence under green light (460-550 nm). It’s obvious to see that the R-CDs can pass through the cell wall into the cell and reach the nucleus. The cell wall and nucleus are clearly visualized which indicates that the CDs can be used as a bio-imaging agent. We can also see that there exists pink light emission in certain cells. However, CDs contains large number of functional groups, such as -OH and -COOH. Usually, CDs are sensitivity to the pH of their micro-environment. When confronted with acid or alkali environment, the fluorescence of CDs will be greatly affected, making its fluorescence red shift or blue shift. Onion epidermis cell structure includes cell membrane, cytoplasm, nucleus, cell wall and vacuole. There are a variety

Fig. 8. Fluorescence microscopic images of onion epidermal cells under different excitation light irradiation were labeled. (a) Ultraviolet light exposure, (b) violet light exposure, and (c) green light exposure, respectively.
of substances dissolved in the cell fluid within the vacuoles, and the pH values within each cell could vary. Within a specific pH change, the functional group can be altered and results in a change in fluorescence intensity and wavelength, e.g., the pink light emission [44]. Therefore, CDs can also be used as fluorescent probe to detect cell variation.

In Fig. 9, we can see that the activity of BEAS-2B depends sensitively on the CDs concentration. The cell activity decreases with increasing CDs concentration. When the concentration of CDs is less than or equal to 0.08 mg/mL, the cell activity is almost not affected. However, when the concentration of CDs was great than or equal to 1.38 mg/mL, the cell activity is below 70% as shown in Fig. 9(a). Moreover, BEAS-2B are grown to a sufficient amount great than 1000 in a cell culture flask and transplant them into a laser confocal dish for culture. After the cells stuck to the wall, the medium is sucked out and adding the CDs solution with a concentration of 0.34 mg/mL with the complete medium solvent. After 4 hours, the bright field image of BEAS-2B measured by a Leica TCS SP8 STED laser confocal microscope is shown in Fig. 9(b). Fig. 9(c) shows fluorescence imaging of BEAS-2B of the same sample by laser excitation with wavelength of 405 nm. Fig. 9(d) is the microscopic image of BEAS-2B with the combination of Fig. 9(b) and (c). We can obtain that the BEAS-2B could still have good cell activity and fluorescence property with an appropriate CDs concentration. Meanwhile, it had been shown that positively
charged CQDs are more cytotoxic and have lower photoluminescence (PL) compared to negative CQDs [45]. After CDs passivation, its surface positive charge is reduced and its toxicity is reduced too.

4. Conclusions

In this study, the fluorescent CDs were extracted from cigarette smoke and passivated by sodium borohydride to enhance the fluorescence of the CDs. The CDs were applied to improve the imaging quality of biological cells. The average size of the core aromatic structure region of $sp^2/sp^3$ carbon is about 3 nm and its edge contains abundant hydroxyl, carbonyl, carboxyl and other oxygen-containing functional groups. Under the excitation wavelength of 360 nm, the fluorescence peak wavelength is 456 nm and the fluorescence quantum yield is 6.13%. The fluorescence intensity of R-CDs obtained by reducing CDs with sodium borohydride was increased by about 7 times and the fluorescence yield was increased to 8.86%. Meanwhile, the fluorescence peak has red-shift to 463 nm. When R-CDs drops were added to the surface of onion epidermal cells and absorbed naturally, it could be clearly observed that the cell wall and nucleus emit bright fluorescence from R-CDs. CDs also show low toxicity to BEAS-2B with good biological activity. These results indicate that CDs and R-CDs have good biocompatibility and fluorescence properties which can improve the imaging quality of biological cells.

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**Data Availability Statement.** Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

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