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Green synthesis of fluorescent carbon dots from carrot juice for in vitro cellular imaging

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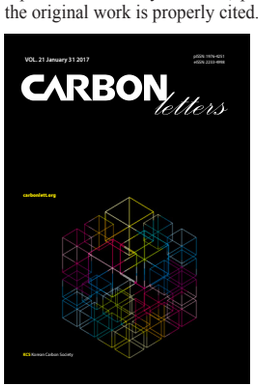

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Abstract

We report the use of carrot, a new and inexpensive biomaterial source, for preparing high quality carbon dots (CDs) instead of semi-conductive quantum dots for bioimaging application. The as-derived CDs possessing down and up-conversion photoluminescence features were obtained from carrot juice by commonly used hydrothermal treatment. The corresponding physiochemical and optical properties were investigated by electron microscopy, fluorescent spectrometry, and other spectroscopic methods. The surfaces of obtained CDs were highly covered with hydroxyl groups and nitrogen groups without further modification. The quantum yield of as-obtained CDs was as high as 5.16%. The cell viability of HaCaT cells against a purified CD aqueous solution was higher than 85% even at higher concentration (700 μg mL^{-1}) after 24 h incubation. Finally, CD cultured cells exhibited distinguished blue, green, and red colors, respectively, during in vitro imaging when excited by three wavelength lasers under a confocal microscope. Offering excellent optical properties, biocompatibility, low cytotoxicity, and good cellular imaging capability, the carrot juice derived CDs are a promising candidate for biomedical applications.

Key words: carbon dots, carrot, fluorescent, biocompatibility, bioimaging

1. Introduction

Fluorescent carbon nanodots (CDs), an intriguing carbon nanomaterial, have been extensively applied in optoelectronic devices, photocatalysis, electrocatalysis, biosensing, and bioimaging owing to their unique structural features and excellent optical and biochemical characteristics [1-4]. Compared with conventional semiconductor-based quantum dots, CDs offer better biocompatibility, water dispersibility, environmental friendliness, non-toxicity, and lower cost [1,5]. Furthermore, on the basis of their excitation-dependent photoluminescence (PL) properties, they are excellent optical nanoprobes candidates for bioimaging applications. Although the mechanism of the excitation wavelength-dependent PL properties of CDs has not yet been explained, it is generally believed that these properties are related to their unique surface energy trap structure containing a graphitized sp^2 in the core and abundant functional groups in the shell [3,6,7].

Generally, CDs can be synthesized by either "top-down" or "bottom-up" approaches [8]. The first category involves breaking larger graphitic materials into small CDs, and methods include use of arc-discharged soot [9], laser ablation of carbon targets [10], electrochemical shocking of carbon nanotubes or electrochemical exfoliation of graphite [11,12], and chemical oxidation [13]. In second approach CDs are obtained from molecular precursors

via chemical synthesis, such as chemical or thermal oxidation of suitable chemical precursors [14,15], ultrasonic treatment of alkali or acid aqueous solutions of glucose [16,17], and microwave pyrolysis of carbohydrates [18,19]. By contrast, the hydrothermal method is considered to be a facile, green, and efficient approach. Recently, natural biomass precursors as raw materials (such as glucose, konjac, chitosan, potato, *Prunus mume* fruit, cabbage, egg, grass, coffee, and others) for the preparation of functional CDs via the hydrothermal method have gained popularity due to the abundance of these carbon sources, as well as their availability, low cost, and eco-friendliness [14,20-28]. In this regard, the preparation of high quality CDs from a suitable biomass as a carbon source is essential.

Herein, we report the use of carrot as a new and inexpensive biomaterial source to prepare high quality CDs instead of semiconductive quantum dots for bioimaging applications. Benefiting from a one-step low temperature hydrothermal technique, the suggested approach is expected to pave the way for large-scale preparation of eco-friendly CDs with multi-functional groups. The surfaces of the obtained CDs were highly covered with hydroxyl groups and nitrogen groups without further modification. Furthermore, the cytotoxicity and cellular imaging of the CDs were investigated in detail. CD cultured human epidermal keratinocytes (HaCaT) cells exhibited distinguished blue, green, and red colors, respectively, during in vitro imaging when excited by three wavelength lasers under a confocal microscope.

2. Experimental

2.1. Materials and characterization

Carrots were purchased from a local market and used as received. Distilled water was used through the whole study. Carrot juice was prepared by a juice extractor, and a dialysis bag was purchased from Spectrum, Inc., USA.

The morphology and the size of the as-obtained CDs were determined using transmission electron microscopy (TEM; JEM-ARM-200F, JEOL, Japan). The crystallinity of the CDs was tested by X-ray diffractometry (Rigaku Co., Japan). Fourier transform infrared (FTIR) spectra of the samples were recorded using a Varian 1000 Scimitar series spectrometer. X-ray photoelectron spectroscopy (XPS) was investigated on an ESCALAB MK-II (VG Scientific Co., USA). Ultraviolet-visible (UV-vis) absorption spectra were obtained with a Shimadzu UV-2600 spectrophotometer, Japan. Photoluminescence excitation and emission spectra were obtained on a LS55 Fluorescence Spectrometer (PerkinElmer, USA). Multicolor cell images were obtained using a LSM 510META confocal laser scanning microscope (CLSM; Carl Zeiss, Germany).

2.2. Synthesis of fluorescent CDs

A clean carrot was cut up and minced for 5 min in a fruit-juicer with 200 mL ultrapure water. After collecting the juice by filtration, 30 mL of carrot juice was transferred into a hydrothermal reactor. The reactor was then operated at 160°C for 6 h and cooled naturally before opening. The raw solution was subsequently centrifuged at 8000 rpm for 30 min to remove large particles and scorched solids. The supernatant was introduced to dialyze in pure water using a tubular dialysis bag (Molecular Weight Cut-off (MWCO)~1 kDa) over night. Finally, the purified CDs aqueous dispersion was freeze-dried and collected, and the CD powder was re-dispersed in metered volume and stored at 4°C for further use.

2.3. Cytotoxicity assay

HaCaT cells were selected and cultured at 37°C, 5% CO_2 and 95% humidity, and a serum-free Keratinocyte-serum free medium basal medium supplemented with 10% fetal bovine serum, penicillin (100 IU mL^{-1}), and streptomycin (100 μg mL^{-1}) was adopted [21].

The cytotoxicity of the CDs was evaluated by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Different levels of CD aqueous solution (20, 100, 300, 500, 700, and 1000 μg mL^{-1}) were incubated together with HaCaT cells for 24 h. After incubation, the individual cells were treated with 20 μL 5 mg mL^{-1} MTT solution. The upper solution was removed and the formazan was dissolved in dimethyl sulfoxide before checking the UV-vis absorbance.

2.4. Cell imaging experiments

The HaCaT cells were incubated in a culture medium containing 500 μg mL^{-1} CDs for 24 hours under the same controlled culture environment as used for the cytotoxicity assay, after which the cells were washed with the phosphate-buffered saline buffer solution and captured on the confocal fluorescence microscope.

3. Results and Discussion

3.1. Synthesis of CDs

The carbon dots were obtained after hydrothermal treatment, centrifuging, and dialysis (Fig. 1). Carrot juice was employed as a natural carbon source to be used in the hydrothermal method, which is a generally acceptable approach that is frequently used in calcination of natural sources. The purified CD aqueous dispersion exhibited vivid cyan emission under UV 365 nm wavelength whilst it showed slight yellow under natural light. The quantum yield was calculated as 5.16%, and quinine sulphate was used for the determination of the QY as the standard reference [21].

3.2. Physiochemical characterization

The morphology and size distribution of these CDs are shown in TEM micrographs (Fig. 2a), revealing that many small carbogenic nanoparticles were well separated from each other. The average diameter was 5.5 nm and they had a narrow size distribution ranging from 3 to 8 nm. The HR-TEM image also showed clear lattice spacing with an interfringe distance of 0.345 nm, which may be attributed to the (002) facet of the sp^2 graphitic crystal phase of graphene [29]. Most of these CDs were amorphous but a typical X-ray dif-

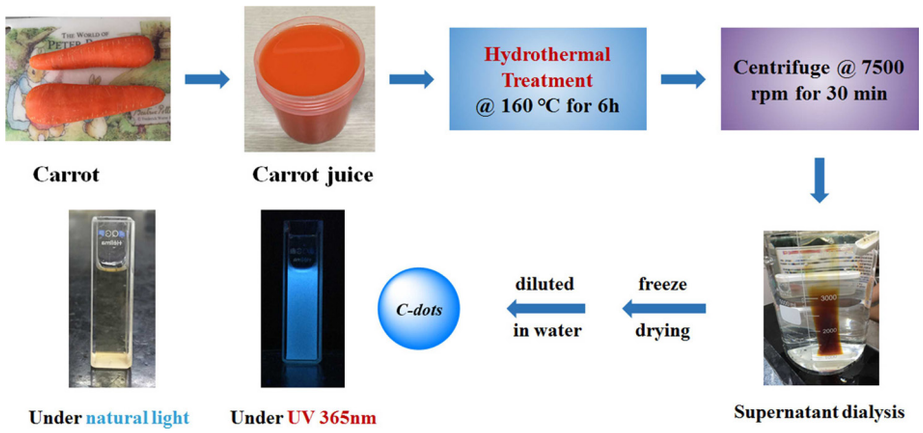

Fig. 1. Schematic illustration of the preparation and purification procedure of as-obtained carbon nanodots (CDs) and an aqueous dispersion of the CDs exposed under natural and ultraviolet (UV) 365 nm light.

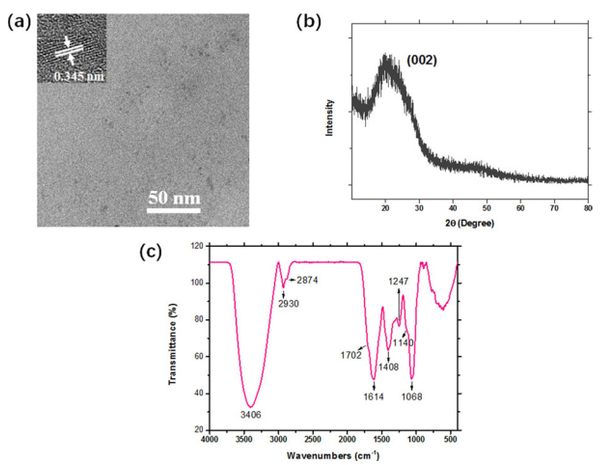

Fig. 2. (a) Transmission electron microscopy images of carbon nanodots (CDs), (b) X-ray diffraction pattern, and (c) Fourier transform infrared spectrum of the as-prepared CDs.

fraction pattern of the resultant CDs is displayed in Fig. 2b. A broad diffraction peak centered at around 22.0° appeared due to the small size of the as-obtained CDs, and it corresponded to the (002) lattice spacing of carbon materials [30]. FT-IR spectra of as-prepared CDs are presented in Fig. 2c. Noticeably stronger peaks emerged at 3406 cm^{-1}, which may correspond to the stretching vibrations of O–H and N–H. A variety of peaks at 1702, 1614, 1408, and 1247 cm^{-1} were assigned to the stretching vibrations of C=O in ketone, stretching vibrations of C=C and C=N, and bending vibrations of −OH, respectively [21,29,30]. These results indicated that the as-prepared CDs possessed abundant functional groups, which is beneficial for enhancing their PL property.

To further analyze their chemical structure, XPS spectra were collected and are provided in Fig. 3. The CDs were composed of carbon (65.98%), nitrogen (2.08%), and oxygen (31.95%). The C 1s spectrum in Fig. 3b can be divided into four distinct carbon states, at 284.3, 285.8, 286.9, and 287.8 eV, which were ascribed to C=C or C–C, C–OH or C–O–C, C–N, and C=O, respectively [21,30]. Similarly, the three peaks for N 1s (Fig. 3c) were three kinds of N atoms associated with C=N–C (397.8 eV); N–$(C)_3$ (399.3 eV), and N–H/NH_2 (400.4 eV) [26]. The O 1s spectrum shown in Fig. 3d exhibited two peaks, at 531.4 and 532.4 eV, which were assigned to the C=O and C–OH/C–O–C bands, respectively [30]. The N–H and C=O bonds presented by XPS suggested the formation of amide bonds, consistent with the FT-IR results, which indicated functionalization for synthesized carbon quantum dots of hydroxyl, carboxylic, and amino groups during the hydrothermal treatment.

3.3. Optical characterization

As shown in Fig. 4a, the UV-vis curve showed two absorption peaks located at around 254 nm and 320 nm, which were ascribed to π-π* and n-π* transitions, respectively, and this feature was similar to that seen in other reported CDs [22,27]. The maximum PL excitation wavelength was at around 360 nm while the maximum emission was at 442 nm, showing a general Stoke Shift of 82 nm. The CD aqueous dispersion displayed an excitation dependent down-conversion fluorescence character. Specifically, the PL emission intensity showed a step-by-step decrease when excited from 360 nm to 520 nm wavelength, accompanied by the emission position shifting from 442 nm to 565 nm, as shown in Fig. 4b. The normalized PL intensity curves also are presented in Fig. 4c. The excitation dependent PL phenomenon might be caused by emissive traps and surface states, which resulted from abundant self-passivated oxygen and nitrogen-containing functional groups (Fig. 3) on the surface of CDs [6,7], agreeing well with other reported CDs prepared from natural sources [4,14,15,27,28].

Interestingly, the CD aqueous dispersion showed an up-conversion PL emission (Fig. 4c), and a growing signal around 475 nm appeared together with a red-shift when excited from 675 nm to 800 nm wavelength (Fig. 4d). These results indicated the as-obtained CDs possess an excitation independent up-conversion feature, which is desirable for biomedical applications, and thus the as-obtained CDs are a promising candidate for cell labelling,

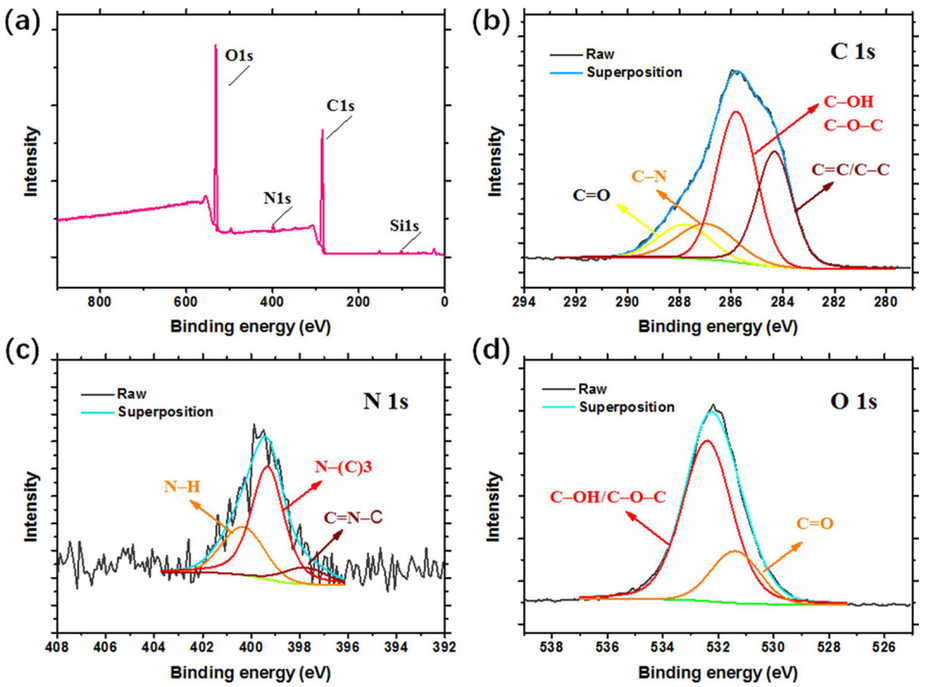

Fig. 3. X-ray photoelectron spectroscopy spectra of carbon nanodots: (a) survey, (b) C 1s, (c) N 1s, and (d) O 1s spectra.

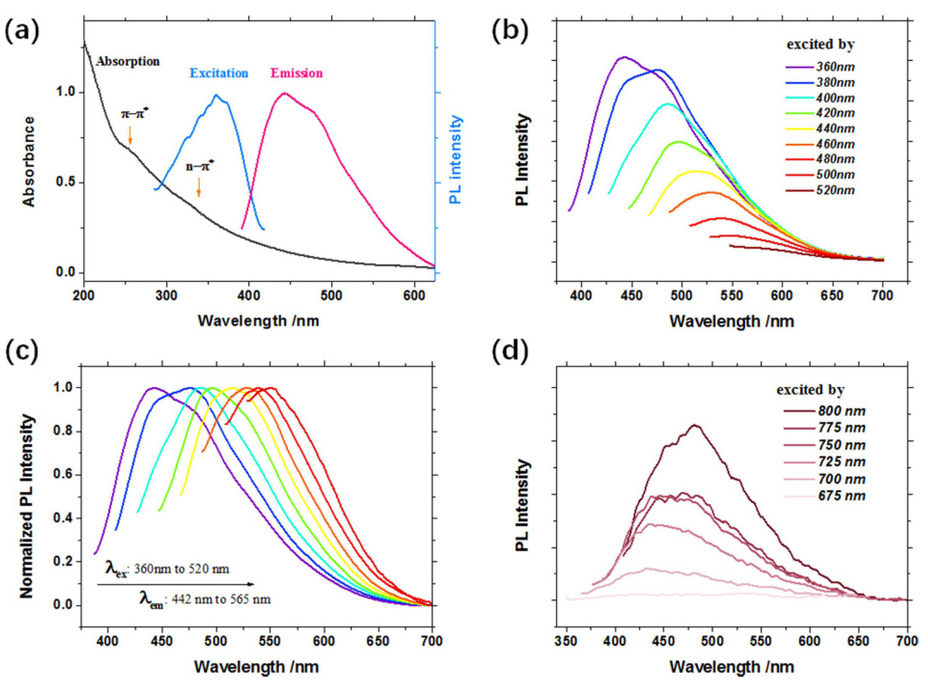

Fig. 4. Optical properties of the carbon nanodots (CDs): (a) Ultraviolet-visible absorption, photoluminescence (PL) excitation, and emission spectra of as-obtained CDs aqueous dispersion. (b) PL emission spectra of CDs dispersion excited sequentially from 360 to 520 nm wavelengths. (c) Normalized PL emission spectra from 442 to 565 nm. (d) Up-conversion PL emission spectra excited from 675 to 800 nm.

imaging, and even drug delivery applications [3,28].

3.4. Cytotoxicity test and cellular imaging

Low cytotoxicity is one of the most important requirements for bio-imaging agents employed in biomedical areas. Hence, to realize potential cytotoxicity of the as-obtained CDs, an MTT assay was carried out using HaCaT cells. The cell viability results of human HaCaT cells against CDs were obtained after the mixture was incubated at arranged concentrations from 0 to

1000 μg mL^{-1} for 24 h, as shown in Fig. 5.

The results showed that the cell activity was still more than 85% after 24 h treatment by a carrot-derived CD solution at a concentration of 0–700 μg mL^{-1}, and the cell viability also did not decrease remarkably even when a 1000 μg mL^{-1} dose of CDs was employed. These observations clearly indicated that our CDs are a viable candidate for cellular imaging in biomedical applications.

Cellular imaging was performed by using HaCaT cells as well as carrot-derived CDs with an administered dose of 500 μg mL^{-1}, and the imaging results were recorded by a confocal fluorescence microscope.

As shown in Fig. 6, the untreated HaCaT cells (Fig. 6a-c) did not show emission color images when excited by the 405, 488, and 543 nm laser, respectively, while CD treated cells showed distinct, sensible multicolor images when several individual HaCaT cells were excited under a confocal microscope with the same conditions (Fig. 6d-f), which could contribute to the excitation dependent PL property of the as-prepared CDs. The results clearly revealed that the carrot-derived CDs are a desirable label agent, and the CDs penetrated both the cell membrane and the plasma but not the nucleus. These results suggest that our low-toxicity, biocompatible CDs derived from a natural, green source have promise for application in cell labelling, imaging, and other biomedical fields.

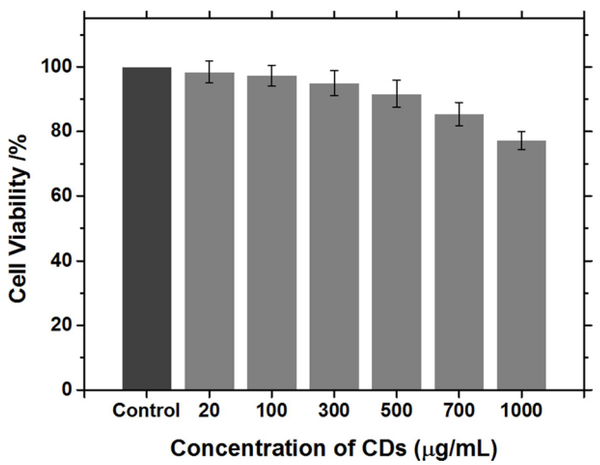

Fig. 5. Cell viability assay of human HaCaT cells against carbon nanodots (CDs) at arranged concentrations from 0 to 1000 μg mL^{-1}.

4. Conclusions

Carrot juice was employed as a novel natural source in conjunction with the commonly used hydrothermal method to derive carbon dots possessing down and up-conversion PL features. The physiochemical and optical properties of CDs were investigated by electron microscopy, fluorescent spectrometers, confocal laser scanning microscopy, and other spectroscopic methods. The quantum yield of as-obtained CDs was higher up to 5.16%. The cell viability of HaCaT cells was higher than 85% even at higher concentration (700 μg mL^{-1}) after 24 h incubation against the purified CD aqueous solution. Eventually, CD cultured cells exhibited multiple blue, green, and red colors, respectively, when excited by three wavelength lasers under a confocal microscope during in vitro imaging. The excellent optical properties, biocompatibility, and low cytotoxicity as well as good cellular imaging capability of the carrot juice-derived CDs are highly desired in biomedical applications.

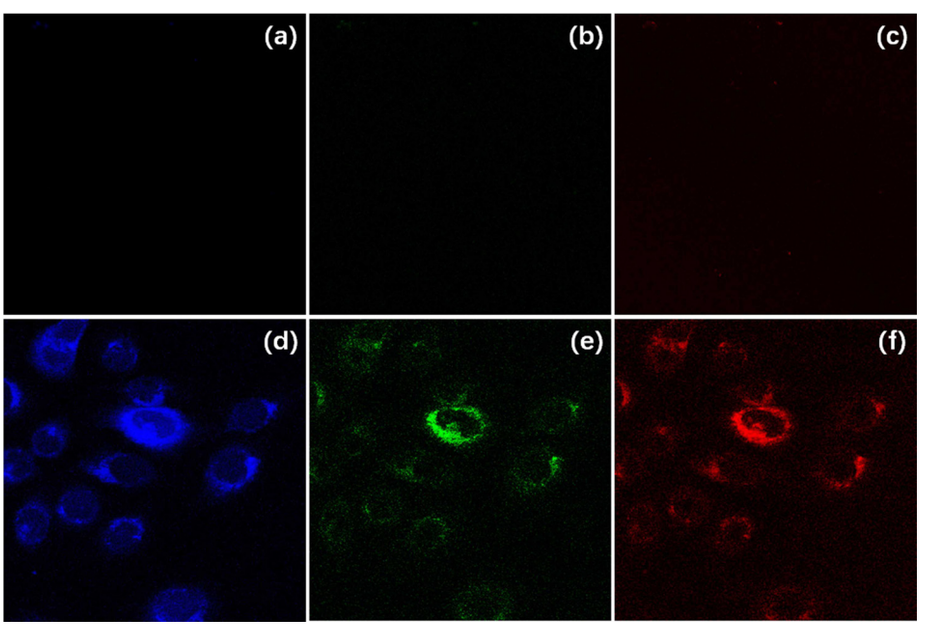

Fig. 6 Untreated HaCaT cells (a-c) images recorded by a confocal fluorescence microscope as well as treated cells (d-f) with carbon nanodots at a concentration of 500 μg mL^{-1} excited by 405 nm, 488 nm, and 543 nm lasers, respectively.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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