DOI: 10.1557/adv.2019.12

Low-cost synthesis of carbon nanodots from millets for bioimaging

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ABSTRACT

We presented a green and simple method to synthesize carbon nanodots (C-dots) from millets using hydrothermal synthesis route for the first time. The obtained C-dots have average diameter ranging from 6 to 10 nm. Optical measurements showed the insight into the formation of functional groups on the particle surfaces, resulting in their good water solubility and bioconjugation. After treatment with C-dots, small subpopulation of the human cervical tumor cells became bright and exhibited multicolor fluorescence under different excitation wavelength. The achievement demonstrated potential applications of fluorescent C-dots in the field of biomedical application.

INTRODUCTION:

Inorganic semiconductor quantum dots have many intriguing features and are currently intensively researched [1]. In term of biomedical application, quantum dots create expected effects, such as bright fluorescence, high biocompatibility, excellent photostability and so forth [1-3]. Among new generation of quantum dots, carbon quantum dots (C-dots) display many interesting features and are considered as a fascinating substitute for traditional semiconductor quantum dots [4-7]. In this context, C-dots possess not only advantageous characteristics of semiconductor quantum dots but also low cytotoxicity [4, 8].

Up to now, there is a large number of approaches to fabricate C-dots from chemical reagents as well as natural biomass [9-12]. Consequently, hydrothermal

synthesis route is considered as favourable method because of its inexpensive apparatus and low energy consumption [12]. Furthermore, in the effort to improve the eco-friendly approaches, natural biomass provides an ideal source for scientists to prepare C-dots. Several results using hydrothermal strategy for the preparation of C-dots from natural bioresources have been reported [9, 13-15]. Concerning to millets, the carbohydrate in total main nutrient is even up to 70% and it is could serve as a great carbonaceous precursor for C-dots synthesis [16, 17].

In our study, we report a low cost and green synthesis of C-dots from millets by hydrothermal treatment for the first time. Optical measurements was performed to provide information on excitation wavelength dependence of C-dots as well as the functional groups on surface of carbon core. In particular, we attempted to examine bioconjugation by incubating human cervical cancer cells with the obtained C-dots. It would provide us information in adjustment of the C-dots synthesized from millets for bioimaging applications.

EXPERIMENTAL DETAILS

Synthesis of C-dots

The dried millets (8.0 g) was first grinded into fine powders and then dispersed into 100 mL of distilled water. The resulting mixture was then transferred into a 100 mL Teflon-lined autoclave and heated at 200^{o}C for a period of 4 h in an oven. After cooling down to room temperature naturally, the brown black carbonized solution were roughly purified through a 100 µm filter paper. The C-dots solution was extracted by centrifugation at 14000 rpm for 10 minutes to remove the large particles. Finally, the obtained C-dots were stored at 4^{o}C for future use.

Cell culture and bioimaging observation

Human tumor specimen was isolated from a 50 year-old woman diagnosed with cervical cancer. It was provided by Phan Thi Thuy Hoa, Ph.D., Hue Central Hospital, Vietnam. We firstly washed the tumor three times with PBS and 1% antibiotics (Streptomycin, Penicillin, Gentamycin). The specimen was then removed from unrelated fibrous tissues and cut into small pieces in the size of about 3 mm^2. After incubating in Trypsin/EDTA 0.25% for 20 minutes, we again removed unrelated fibrous tissue and added Dulbecco's Modified Eagle's Medium (DMEM) into the samples. By centrifuging at 2500 rpm for 5 minutes, the obtained cells were extracted and maintained with the density of 10^4 cells/cm^2. In this work, cervical cancer cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum (FBS), 1% glucose and antibiotics at 37°C in 5% CO_2 for 24 h [18, 19]. Finally, the cells were grown in medium containing 15% C-dots and washed three times with PBS before fluorescent microscope observation.

Instrument

We characterized the optical properties of the obtained C-dots by carrying out photoluminescence (PL) on a Fluorolog FL-22 (Horiba, Japan), ultraviolet–visible absorption spectra on GENESYS 10S UV-Vis (Thermo Scientific, American), Fourier-transform infrared (FTIR) spectroscopy on FTIR Affinity-1S (Shimadzu, Japan). Fluorescence imaging studies were performed with an optical microscope Leica DM2500

(Leica, Germany) and the specimens were excited by halogen lamp with wavelength at 488 nm and 561 nm, respectively. The size and morphology of the C-dots were observed by using a transmission electron microscopy JEOL JEM–1010 (JEOL, Japan) with an accelerating voltage of 80 kV. For structural characterization, X-Ray diffraction (XRD) of were recorded on a D8 Advance (Bruker, Germany) in the range from 1° to 70°.

RESULTS AND DISCUSSION

Figure 1A show the transmission electron microscopy (TEM) image of the obtained C-dots. Roughly, the size histogram of C-dots depicted in the upper left corner of Figure 1A indicated that the C-dots were mainly distributed in the range of 6–10 nm. The result agree with previous publications [13-15, 20]. Together, the X-ray diffraction pattern of the C-dots shown in Figure 1B illustrated a broader peak at 2θ~21°, revealing an amorphous nature of C-dots phase. This evidence is attributed to the (002) lattice spacing of disorder carbon atoms [21, 22].

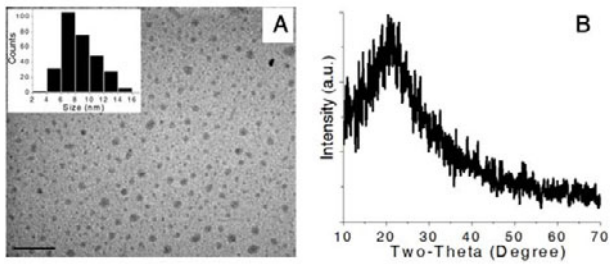

Figure 1. (A) TEM image of the obtained C-dots with the scale bar is 500 nm and the inset shows the corresponding dot size distribution. (B) The XRD pattern of obtained C-dots.

To investigate the optical properties of C-dots, the UV–vis absorption and emission spectra of the C-dots with different excitation wavelength were recorded. As pointed out in Figure 2A, the UV–vis spectrum showed a shoulder peak centered at 280 nm along with a shoulder extending into the visible range, corresponding to π-π* transition of the C=C bonds [18, 22]. The inset photograph in Figure 2A displays the optical images of the C-dots under sunlight and UV light illumination (410 nm), respectively. The bright green photoluminescence (PL) of the C-dots is strong enough to be seen with the naked eyes. To further clarify optical behaviour of C-dots, we examine phenomenon of excitation dependent PL. As shown in Figure 2B, by changing excitation wavelength from 320 to 480 nm (in 20 nm increments starting from 320 nm), the PL spectra are broad and ranging from 360 (violet) to 500 nm (green). Namely, the emission peak position shifts to the longer wavelength and the PL intensity tend to decreases as the excitation wavelength is increased. However, the difficult issue is to estimate the role of carbon core and functional groups with respect to the PL spectra. The intriguing excitation wavelength dependence of C-dots has not yet been explained clearly. The possible origin could result from the optical selection of surface state owing to hybridization of the carbon core and the functional groups [7, 12, 15, 23].

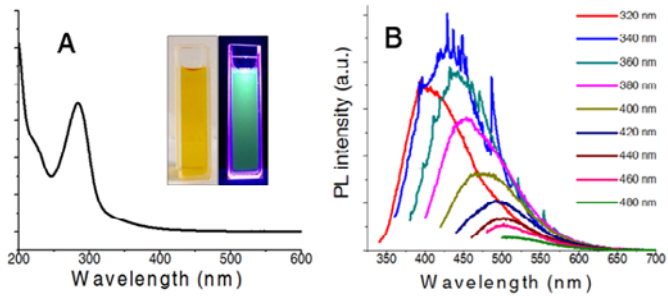

Figure 2. (A) UV–vis absorption spectra of C-dots show peak centered at 280 corresponding to π-π* transition of the C=C bonds. Insert photograph displays the optical images of the C-dots under sunlight and UV light illumination (410 nm), respectively. (B) The PL spectra of obtained C-dot under different excitation wavelength from 320 to 480 nm (in 20 nm increments starting from 320 nm).

Since functional groups play a very important role with respect to bioconjugation, we performed FTIR spectroscopy to further clarify the surface of carbon core. The FTIR spectroscopy shown in Figure 3 displays the characteristic absorption bands of an O–H stretching vibration at 3444 cm^{-1}, C–H stretching vibration at 2958 and 2931 cm^{-1}, C=O stretching vibration at 1774 cm^{-1} and N–H bending vibrations at 1741 and 1658 cm^{-1} [12, 13, 24]. Several peaks ranging from 1200 to 1000 cm^{-1} (1201 cm^{-1}, 1118 cm^{-1}, 1014 cm^{-1}) result from the vibration mode of C-O [25]. The peak at 1400 cm^{-1} is attributed to a–CH_2–stretching vibration deformation [13]. According to the above-mentioned assignments, it indeed reflects that the functional groups, including hydroxyl, carbonyl/carboxyl, amino were introduced onto the surfaces of the C-dots during the thermal reaction. The results promoted us in further investigate bioimaging of obtained C-dots.

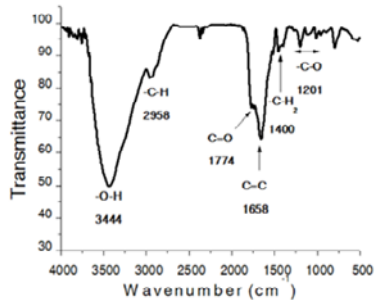

Figure 3. FTIR spectrum of obtained C-dots reflects the functional groups, including hydroxyl, carbonyl/ carboxyl, amino were introduced onto the surface of the C-dot.

Fluorescent images of human cervical cancer cells incubated for 24 h with the obtained C-dots are shown in Figure 4. During the culture process, growing cancer cells appeared as locally restricted and constructed small subpopulation of the tumor cells (marked with red circles). As shown in Figure 4B and 4C, cancer cells exhibited

fluorescence including yellow and red colors under 488 nm and 561 nm excitation wavelength while the control sample (without C-dots) was silent. The result are clearly consistent with the previous publications [18, 26, 27]. The dyed cancer cell is still being studied, the possible origin could result from bioconjugation between C-dots and specific proteins produced by cancer cells [4, 23].

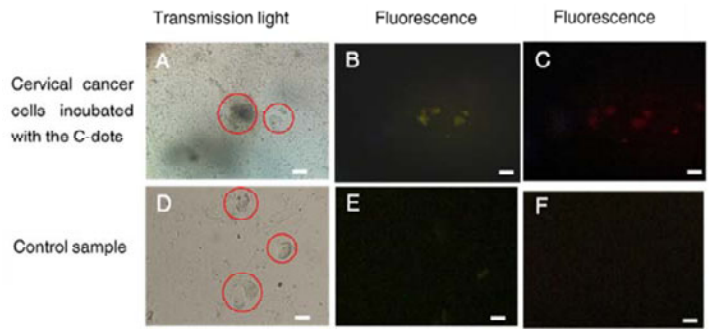

Figure 4. Optical microscopy of human cervical cancer cells (magnification 40x). Cervical cancer cells incubated with the obtaind C-dots (A) and control sample (D) under transmission light and at excitation wavelength of 488 nm (B, E) and 561 nm (C, F). Scale bar is 30 µm.

CONCLUSION

We fabricated C-dots from millets using hydrothermal synthesis. The green and low cost obtained C-dots exhibited bright fluorescence under different excitation wavelength. The first investigation of bioimaging with human cervical cancer cells indicated the bioconjugation of C-dots. Obviously, the result showed that the obtained C-dots could be employed as a solution for bioimaging owing to its bright and multicolor luminescence. The achievements suggest further investigation in both photoluminescence and imaging applications.

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