HYDROTHERMAL SYNTHESIS OF CARBON NANODOTS FROM Vigna radiata FOR BIO-IMAGING IN VITRO

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SUMMARY

The fluorescent C-dots have great potential applied in photocatalysis, bio-imaging and other related areas. In this study, we present a green, simple and economical method to synthesize carbon nanodots (C-dots) from mung bean (Vigna radiata) using hydrothermal synthesis approach. They overcome the shortcomings of high toxicity of traditional nanomaterials. C-dots from Vigna radiata are a type of fluorescent nanomaterials, which not only possess the specific quantum confinement effects of nanomaterials due to the small size of nanomaterials, but also have good biocompatibility and high fluorescence. The obtained C-dots have average diameter ranging from 6 to 8 nm. Optical measurements showed the formation of hydroxyl, carbonyl/carboxyl, amino functional groups on the particle surfaces, resulting in their high hydrophilicity and bioconjugation. After incubation with C-dots, the SK-LU-1 cells became bright and exhibited multicolor fluorescence under different excitation wavelength. The fluorescent C-Dots from Vigna radiata possess rather strong ability to bind with other organic and inorganic molecules due to their abundant surface groups, so that fluorescent C-Dots Vigna radiata can be manipulated via a series of controllable chemical treatments and satisfied the demands in the protocol fluorescent staining of cells. The achievement demonstrated potential applications of fluorescent C-dots in the field of biomedical application, especially in diagnostic disease techniques.

Keywords: Carbon nanodots, Photoluminescence, Natural biomass, Vigna radiata, Hydrothermal method, Biomedical application, SK-LU-1 cells.

INTRODUCTION

Inorganic semiconductor quantum dots have many intriguing features and are currently intensively researched. In term of biomedical application, quantum dots create expected effects, such as bright fluorescence, high biocompatibility, excellent photostability and so forth (He et al., 2008; Murray et al., 1993; Xiaohu et al., 2005). Among new emerging quantum dots, carbon quantum dots (C-dots) exhibit many interesting features and are considered as a fascinating substitute for traditional semiconductor quantum dots (Haitao et al., 2012; Pooria et al., 2017; Zhi et al., 2013). In this context, C-dots not only possess advantageous characteristics of semiconductor quantum dots but also are believed to be a kind of low nanotoxicology owing to free toxic heavy metal (Sheila, Gary, 2010).

Up to now, there is a large number of approaches to fabricate C-dots from chemical reagents as well as natural biomass (Haitao et al., 2011; Jingyi et al., 2013). Consequently, hydrothermal synthesis route is considered as favorable method because of its inexpensive apparatus and low energy consumption (Jumeng et al., 2014). Furthermore, in the effort to improve the eco-friendly approaches and replace chemical reagents for synthesizing nanomaterials, natural biomass provides an ideal source for scientists to prepare C-dots with deliberated properties. Several results using hydrothermal strategy for the preparation of C-dots from natural bioresources have been reported (Chengzhou et al., 2012; Wenbo et al., 2012; Yingshuai et al., 2014).

Mung bean (Vigna radiata) possesses valuable properties such as keeping low blood sugar, helping steady blood LDL, activating antioxidant, and retaining the total carbohydrate up to 62.62%, those make mung bean become a great carbonaceous precursor for C-dots synthesis.

The work shows that C-dots emerge good water-solubility and emit bright and colorful fluorescence under different excitation wavelength. Remarkably, we have investigated bioconjugationof C-dots by incubating the SK-LU-1 cells with C-dots. The results demonstrated it as an efficient fluorescent probe for bioimaging applications.
MATERIALS AND METHOD

Experimental procedure for synthesis of C-dots

Mung bean (Vigna radiata) was collected in Hue city, Thua Thien province. The sample was tested and classified by the Plant Department, Department of Biology, University of Sciences, Hue University.

The dried bean Vigna radiata (8.0 g) was first ground into fine powders and then dispersed into 100 mL of distilled water. The mixture is then transferred into a 100 mL Teflon-lined autoclave and heated at 120°C for 4 h in an oven.

After cooling down to room temperature naturally, the brown black carbonized solution was roughly purified through a 70 μm filter. The C-dots solution was extracted by centrifugation at 14000 rpm for 10 minutes to remove the large particles, removing the supernatant using suction. Finally, the obtained C-dots were stored at 4°C for future use. Regarding their surface chemistry, that the C-dots is typically attached with many carboxylic acid moieties, hand ideally imparts them with excellent water solubility, facilitating their purification and characterization.

Application in cell testing

The SK-LU1 is a lung adenocarcinoma cell line that displays epithelial morphology and grows in adherent culture in vitro. This cell line does not form tumors when injected into immunocompromised mice. Chester M. Southam, MD, formerly at Sloan Kettering Institute, Memorial Sloan Kettering. It was provided by Dr. Phan Thi Thuy Hoa, Hue Central Hospital, Vietnam.

The SK-LU1 cells were cultured at the density of 104 cells per cm² in T-flasks 25cm² at 37°C and 5% CO₂ in Dulbecco’s Modified Eagle’s Medium DMEM/12 supplemented with 10% fetal bovine serum (FBS), 1% glucose and antibiotics, in a humidified environment of 95% for 20h (Dubey, 2007).

We conducted cell dying in the condition of chemical components of the culture media supplemented with 15% C-dot supplementation. Wash cells in PBS three times for 3 to 5 minutes and evaluate of results with a fluorescence microscope.

Statistical analysis

All values were expressed as the mean and SEM (Standard Error of the Mean). To compare results between groups, we used a T test or a one-way ANOVA followed by Tukey test with Sigmostat software (Systat Software Incorporation). Significance for all analyses was set at ***P < 0.001 or **P < 0.05.

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 cancer cells 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

Fig. 1(a) show the typical transmission electron microscopy (TEM) image of the obtained C-dots. Roughly, the size histogram of C-Dots depicted in the upper left corner of Fig. 1(a) indicated that the C-dots were spherical and diameter were mainly distributed in the range of 6–8 nm. The result is consistent with previous publications where carbon nanoparticles is distinctively less than 10 nm in size (Jun et al., 2013; Wenbo et al., 2012; Yingshuai et al., 2014). Together, the X-ray diffraction pattern of the C-dots shown in Fig. 1(b) illustrated a broader peak at 20=21°, revealing an amorphous nature of C-dots phase. This evidence is attributed to the (002) lattice spacing of disorder carbon atoms (Rahul, Santanu, 2015; Ramanan et al., 2016).

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 Fig. 2 (a), the UV–vis spectrum showed a shoulder peak centered at 280 nm along with a tail extending into the visible range, corresponding to π–π* transition of the C=C bonds (Ramanan et al., 2016), (Wenbin et al., 2013). The inset photograph in Fig. 2 (a) displays the optical images of the C-dots under sunlight and UV light illumination (410 nm), respectively. The bright green PL of the C-dots is strong enough to be seen with the naked eyes. To further clarify optical behavior of C-dots, we examine phenomenon of excitation dependent PL.

As shown in Fig. 2(b), by changing excitation wavelength from 300 to 480 nm (in 20 nm increments starting from 300 nm), the PL spectra are broad and ranging from 360 (violet) to 500 nm (green). Here, 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 conventional judgment is that excitation-dependent fluorescence could result from the optical selection of surface state owing to hybridization of the carbon core and the functional groups (Jumeng et al., 2016), (Nianjun et al., 2016), (Pooria et al., 2017).

Since they play a very important role with respect to bioconjugation, we performed FTIR spectroscopy to understand the functional groups on the surface of carbon core. The FTIR spectroscopy shown in Fig 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 (Jumeng et al. 2014; Jingyi et al. 2014-13; Swagatika et al. 2012-23). Several peaks ranging from 1200-1000 cm⁻¹ (1201 cm⁻¹, 1118 cm⁻¹, 1014 cm⁻¹) result from the vibration mode of C–O (Moyun C. et al., 2014). The peak at 1400 cm⁻¹ is attributed to a–CH₂–stretching vibration deformation (Jingyi et al., 2013). According to the above-mentioned assignments, it imply that the functional groups, including hydroxyl, carbonyl/carboxyl, amino were introduced onto the surfaces of the C-dot during the thermal reaction. The results promoted us in further investigate cell imaging of obtained C-dots.

To access the potential of C-dot application in some biological fields such as cell dyeing, we tested on the SK-LU1 cells, evaluating the results after 24h of dyeing by LEICA DM2500 fluorescence microscope. Luminescent photosynthesis of tumor cells using C-dot solution in culture medium is depicted in Figures 5. The results of the fluorescence imaging study are fit with the previously published studies (Wang, Liu, 2016). The types of SK-LU1, stimulation with 488 nm and 561 nm wavelengths is strongly emitted in the respective colors of yellow and red. C-dots with tunable photoluminescence from blue to red can be achieved by controlling size distribution by increasing the surface oxidation degree or by adjusting reagents and preparation conditions In the same conditions of culturing and dyeing, its different fluorescence intensity. Exhibit excitation-dependent photoluminescence behavior along with red-shifted of excitation/emission maximum with concentrations C-dots 15%.

The SK-LU1 type of tumor cell produces specific proteins and many of the characteristic features of tumor cells. Alternatively, tumor cells may overexpress chemokine receptors, so the C-dot content goes inside certain cells differently, resulting in the luminous intensity. The mechanism of fluorescence of tumor cells when cultured in a C-dot solution environment can be explained by the C-dot conjugate of specific proteins. Tunable fluorescent applications for the coloration in vitro imaging of carbon nanodots with cells penetration capability is reported photoluminescence the C-dot penetration behavior into cell membrane. The occurrence of proteins such as CK20, Napsin-A, TTF-1, CK7 or some aromatic amino acids in cancer cells allows C-dot to pass through the cell membrane and be cleared when stimulated with Ultraviolet light (Horn et al., 2015).

![Figure 4. FTIR spectrum of obtained C-dots imply the functional groups, including hydroxyl, carbonyl/carboxyl, amino were introduced onto the surfaces of the C-dot](image)

**Figures 5.**

5a. C-dot (Vigna radiata) was dried stimulated by 561 nm
5b. C-dot solution (Vigna radiata) stimulated by 561 nm
5c. In C-dot solution mining (Vigna radiata) on the microscope slide stimulated by 488 nm.
5d. Normal view under a light microscope: SK-LU1 cells are dyed C dot (Red circles) (x40)
5e. Normal view under a light microscope:SK-LU1 cells are dyed Elipticine. (x100)
5f. SK-LU1 cells is stimulated by 488 nm wavelength (x100)
5f. Control sample for SK-LU1 cells are dyed concentrations C-dots 15% stimulated by 488 nm wavelength (X100)
5g.SK-LU1 cells are dyed concentrations C-dots 15% when stimulated by 561 nm wavelengths(X100)
For the samples that aren’t dyed with C-dots, when stimulated by 488 nm and 561 nm wavelengths, we observed that the luminescent light is weak or there is no fluorescent light at all. In the control samples, the area that fluorescence extends over the entire surface of the biological sample, is not the same as the cellular region that exists as the one with-dot sample. So, the material C-dot has passed through the cancer cell membrane and strongly fluorescent with light at 488 nm and 561 nm and the control sample is negative.

CONCLUSION

We fabricated C-dots from \textit{(Vigna radiate)} using hydrothermal synthesis. The obtained C-dots have average diameter ranging from 6 to 8 nm. The green and low cost carbonaceous precursor without passive surface exhibited bright fluorescence under different excitation wavelength wavelength. The first investigation of cell imaging with cells SK-LU-1 indicated the great bioconjugation of obtained C-dot \textit{(Vigna radiate)}, which suggests a potential application in biomedication.

REFERENCES


Như đã biết, quang tương quang của nano C-dots có nhiều tiềm năng ứng dụng trong công nghệ giao tiếp, bệnh nhân sinh học và các lĩnh vực liên quan khác. Trong nghiên cứu này, chúng tôi trình bày phương pháp tổng hợp vật liệu nano C-dots đơn giản, thân thiện với môi trường và tiết kiệm từ hạt đậu (Vigna radiata) bằng phương pháp thủy nhiệt. So với các vật liệu nano truyền thông, vật liệu này khắc phục được khuyết điểm về tồn tại độc tính cao. C-dots từ hạt đậu (Vigna radiata) là một nano thể hiện quang tương quang, sản phẩm không chỉ cung cấp hạt lượng tử có kích thước nhỏ mà còn có tính tương thích sinh học và mức độ quang tương quang tốt. Các C-dots thu được có đường kính trung bình từ 6 đến 8 nm. Các phép đo quang học cho thấy sự hiện diện các nhóm chức hydroxyl, carbonyl / carboxyl, amino trên bề mặt hạt, có tính thẩm nước cao và khả năng tương thích sinh học. Khi ủ với C-dots, các tế bào SK-LU-1 đã thể hiện quang tương quang dưới các bước kích thích khác nhau. Các C-Dots từ hạt đậu (Vigna radiata) có khả năng liên kết mạnh với các phân tử hữu cơ và vô cơ. Vật liệu C-Dots từ hạt đậu (Vigna radiata) có thể thấm gia dieu hòa bể mặt thể bào thông qua một quy trình xử lý hóa học trong quá trình nhuộm và chúng đã thể hiện phát quang trên tế bào SK-LU-1. Kết quả này cho thấy tiềm năng ứng dụng vật liệu C-Dots được tổng hợp từ hạt đậu (Vigna radiata) bằng phương pháp thủy nhiệt trong lĩnh vực ứng dụng y sinh, đặc biệt là trong các kỹ thuật chẩn đoán bệnh.

Từ khóa: Nano cacbon, quang tương quang, vật liệu từ thực vật, hạt đậu xanh, phương pháp thủy nhiệt, ứng dụng y sinh, tế bào ac tính SK-LU-1.

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