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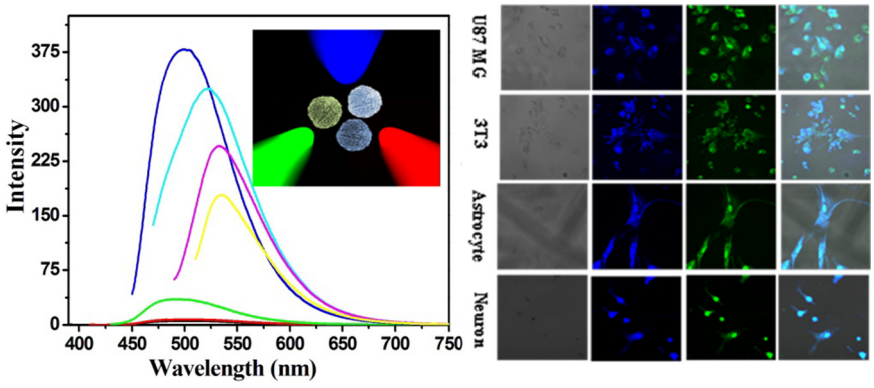
Regular Article

Facile approach to synthesize highly fluorescent multicolor emissive carbon dots via surface functionalization for cellular imaging

Aniruddha Kundu [a], Jungpyo Lee [a], Byeongho Park [a], Chaiti Ray [a], K. Vijaya Sankar [a], Wook Sung Kim [b], Soo Hyun Lee [c], Il-Joo Cho [c], Seong Chan Jun [a,*]

[a] School of Mechanical Engineering, Yonsei University, Seoul 120-749, South Korea
[b] Department of Electrical Engineering, Pohang University of Science and Technology (POSTECH), Pohang 790-784, South Korea
[c] Center for BioMicrosystems, Brain Science Institute, Korea Institute of Science and Technology, Seoul 136-791, South Korea

GRAPHICAL ABSTRACT



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ABSTRACT

Luminescent nanomaterials are encouraging scaffolds for diverse applications such as chemical sensors and biosensors, imaging, drug delivery, diagnostics, catalysis, energy, photonics, medicine, and so on. Carbon dots (CDs) are a new class of luminescent carbonaceous nanomaterial that have appeared recently and reaped tremendous scientific interest. Herein, we have exploited a simple approach to prepare tuneable and highly fluorescent CDs via surface functionalization. The successful synthesis of CDs is manifested from several investigations like high-resolution transmission electron microscopy (HRTEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The CDs exhibit excellent water solubility and with increasing nitrogen content fluorescence quantum yield increases whereas cell toxicity decreases. The CD synthesized at high temperature (180 °C) shows very high quantum yield (more than 56%). The tuneable optical properties of CDs are systematically studied using UV-vis and fluorescence spectroscopy. The cell viability evaluation and *in vitro* imaging study reveals that the synthesized CDs can be employed as a potential fluorescent probe for bio-imaging without further modification.

* Corresponding author.
E-mail address: scj@yonsei.ac.kr (S.C. Jun).

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1. Introduction

A large number of new nanostructured carbon-based materials such as graphene, fullerenes, nano-diamonds, carbon nanotubes etc. have been discovered during the past decades, which exhibit unique physicochemical properties [1]. Recently, a new type of carbonaceous nanomaterial, carbon dots (CDs) also known as carbon nanodots (CNDs) or carbon quantum dots (CQDs) have attracted stubborn scientific attention, since its serendipitous discovery during the purification of single-walled carbon nanotubes (SWCNTs) through electrophoresis by Scrivens and co-workers in 2004 [2]. As a new rising star in the field of luminescent carbon nanomaterials, CDs have harvested much interest as potential candidates to conventional semiconductor QDs. Though they exhibit comparable optical properties, CDs have several advantages over QDs such as low toxicity, excellent water dispersibility, environmental friendliness, high photo-stability and chemical inertness [3–5]. Moreover, simple and inexpensive one step synthesis along with either surface passivation or functionalization of CDs permits for the regulation of their various properties. For that reason, CDs find versatile applications in biological imaging, drug delivery, sensing of metal ions, optoelectronic devices, catalysis, solar cells and so on [6–11]. Recently, our group has studied the optical properties of graphene/functionalized graphene and graphene quantum dots which mainly focused on their luminescent behavior [12–16].

In the past few years, various physico-chemical methods have been explored to prepare CDs, including laser ablation of graphite, arc discharge, electrochemical synthesis, oxidative acid treatment, microwave/ultrasonic methods, thermal oxidation etc [2,17–22]. But, these aforementioned methods involve the use of strong acid, expensive starting materials, sophisticated instruments and complicated processes which limit the applications of synthesized materials. Numerous carbon based materials like graphite, SWCNTs/MWCNTs, candle soot, glycerol, carbohydrate, citrate, polymers are used as starting materials for the synthesis of CDs [23–30]. Thus, for large-scale synthesis of high quality fluorescent CDs simple, environment friendly and sustainable synthetic routes are of potential interest and the hydrothermal method has been exposed to be a facile and effective synthetic approach to produce fluorescent CDs [31]. Recently, polymers are used as surface passivating agent to obtain multi-responsive fluorescence property. For example, hyperbranched polyethyleneimine (PEI) capped fluorescent Ag nanoclusters/CDs are prepared and employed for different sensing phenomenon [32–34]. In this article, branched polyethyleneimine (PEI) functionalized CDs are synthesized using a one-step hydrothermal assisted pyrolysis of citric acid (CA). Here, the formation of CDs and the surface passivation with PEI are achieved simultaneously, utilizing this one pot strategy. We have also thoroughly investigated the effect of temperature and reaction time on the fluorescence property of CDs. The obtained CDs show excellent fluorescence properties and excitation-dependent fluorescence behavior, which will really be helpful for multicolor imaging. Moreover, the CDs synthesized at high temperature exhibit better quantum yield and low cytotoxicity even at high concentration, compared to the CDs obtain at lower temperature. These advantages inspired us to employ as synthesized highly fluorescent and stable CDs as an excellent fluorescent probe for *in vitro* cellular imaging.

2. Experimental sections

2.1. Materials

Branched polyethyleneimine (PEI, Mw = 1200) and citric acid (CA, ACS reagent) was purchased from Sigma Aldrich. Syringe filter (pore size 0.1 μm) was purchased from Sigma Aldrich. All other chemicals used in the cellular experiments were purchased from Gibco, USA and Life Technologies, USA. Deionized (DI) water was used throughout the experiments.

2.2. Synthesis of fluorescent CDs

The fluorescent CDs passivated with branched PEI was synthesized by a facile green route of hydrothermal assisted pyrolysis method. For that purpose, at first equimolar mixture of CA and PEI was dissolved in DI water using different glass vial. Next, these two solutions were mixed and sonicated for 5 min to get homogeneous mixture and transferred to a Teflon-lined stainless steel autoclave chamber. The sealed autoclave was heated with variable temperature (100, 150 and 180 °C) and time was also varied (5, 10, 15 and 20 h) to obtain a vivid idea about the formation of CDs. After the execution of hydrothermal reaction, the clear viscous liquid turned into either pale yellow or bright yellow colour solution depending on the reaction condition, suggesting the formation of carbon dots. The resulted solution was further filtered using a syringe filter to discard large-sized carbon nanoparticles and stored at ambient condition for future study. The CDs synthesized at different temperature are denoted as CD-L (100 °C), CD-M (150 °C) and CD-H (180 °C).

2.3. Quantum yield measurement

The relative fluorescence quantum yield (QY) of CD was measured using quinine sulphate in 0.1 M H_2SO_4 (quantum yield 54%) as a standard [35]. The value of QY was calculated according to the following equation:

$$QY_{sample} = QY_{std.}\left[(I/A)_{sample} \times (A/I)_{std.}\right]\left(\eta^2_{sample}/\eta^2_{std.}\right)$$

where 'A' denotes absorbance at the excitation wavelength and 'η' is the refractive index of solvent. The integrated emission intensity is represented by 'I', which is calculated from the area under the emission peak on the same wavelength scale. To minimize the reabsorption effects at the excitation wavelength, the absorbance values of both sample and standard solutions were kept below 0.1.

2.4. Protocol for cellular study

Here, we have used different cell lines to check the *in vitro* cell viability assay and cell imaging. For instance, human brain tumor cells (U87 MG), standard fibroblast cell (3T3), and two primary cells (neuron and astrocyte) are used. Astrocytes were isolated from postnatal day 1 Sprague-Dawley (SD) rats. On the other hand, the primary rat cortical neurons were isolated from embryonic day 18 Sprague-Dawley (SD) rats. Using neural tissue dissociation KIT (MACS, Miltenyi Biotec, USA), neurons and astrocytes were dissociated.

2.4.1. In vitro cellular imaging

Isolated neurons and astrocytes were seeded respectively into a 24-well culture plate which involves poly d-lysine (PDL) coated round type coverslips at 1.0×10^5 cell per well in Neurobasal cell culture medium (NBM; Gibco, USA) with 2% B27 supplement (Life Technologies, USA), 0.25% Glutamax (Gibco, USA), and 1% penicillin-streptomycin (Gibco, USA). The samples were cultured for 72 h at 37 °C in a humidified 5% CO_2 incubator. Then, neurons and astrocytes were incubated with 100 μg/mL CDs in NBM medium for 2 h. After 3 times washing with phosphate buffered saline (PBS), neurons and astrocyte were fixed with 4% paraformaldehyde for 30 min at room temperature. Finally, cellular fluorescence