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Arginine-modified carbon dots probe for live cell imaging and sensing by increasing cellular uptake efficiency

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ABSTRACT

As a new family of nanomaterials, carbon dots (CDs) have been reported to enable to penetrate plasma membrane and show fluorescence in cells due to their small sizes and fluorescent properties. However, development of CDs as effective cellular imaging probes still remains a challenge, since they have relatively low efficiency of cell permeability which is difficult to be detected by a commonly used fluorescence microscope. Here we introduce arginine-modified carbon dots (Arg-CDs) with strong luminescence that could be used for cell imaging under common fluorescence microscope because of the high cellular uptake efficiency. More interestingly, the cellular luminescence showed red-shifting when the Arg-CDs entered cells, and the red luminescence emission ability depended on the cell lines, including NIH 3T3, HEK 293, Hela and MCF-7 cells. This finding opens up a new application field that the Arg-CDs could be used for cell imaging and sensing simultaneously as a fluorescent probe.

1. Introduction

Despite fluorescent quantum dots (QDs) have been widely used in the field of biomedicine research, such as drug delivery, bio-imaging and sensing [1–3], the concern for biosafety and health risk for the metal-based toxicities has led to the search for alternative nanoparticles [4]. As a new family of nanomaterials, carbon dots (CDs) have drawn considerable attention since their discovery in 2004 [5], due to their favorable biocompatibility and low toxicity. Currently, CDs have been successfully used as safe labeling probes for detecting DNA, metal ions, proteins in vitro based on their impressive properties such as stable photo-luminescence, broad excitation and tunable emission wavelength [6–8].

However, there are several drawbacks in the development of fluorescent CDs as optical imaging probes in live cells [9,10]. For example, synthetic CDs with long emission wavelength always cannot reach reasonable quantum yields (QYs) [11]; the blue or green luminescence possessed by most CDs with short emission wavelength have to overcome the problems of background interferences from organisms in bioimaging [12]. Additionally, the relatively low cellular fluorescence is another drawback for application of CDs in live cell imaging, though it is reported that CDs enable to penetrate plasma membrane and show fluorescence in cells. In order to extend the application of CDs in imaging, a great deal of attempts have been made and the studies mainly focused on the surface functionalization of CDs [13,14]. Positive ligands, including PEI, PEG and organosilane [15–17], have been reported to conjugate with naked CDs for increasing QYs.

Considering most of the synthetic procedures were somewhat lengthy and complicated, we were therefore seeking a simplified strategy to develop CDs as fluorescent probes for cell imaging that could be easily prepared in mild condition and for which the required reagents are readily available. We turned our attention to the basic amino acid—arginine (Arg), the derivatives of which are commonly considered to be able to increase cell uptake, because the positively charged guanidyl group in Arg could electrostatically bind with the negative components plasma membrane, and then promote the subsequent cell endocytosis [18]. We assumed that an arginine modified CDs (Arg-CDs) might have the capability of increasing the cell uptake and then could be used for live cell imaging.

2. Materials and experimental

2.1. Synthesis and characteristic of Arg-CDs

The reaction condition to synthesize the bare CDs was fairly mild compared to the harsh reaction conditions for many other CDs, such as arc discharge, calcining in high temperature, laser ablation [19]. The facile hydrothermal method was employed to prepare bare CDs. Firstly, citric acid (1.057 g) and ethylenediamine (335 μL) were dissolved in distilled deionized water (10 mL), then the solution was heated at reflux

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for 12 h. After the reaction, the resulting solution was cooled down to room temperature. The greenish-black product was further subjected to dialysis (500–1000 kDa dialysis tubing; Spectrum Labs), and the CDs with quantum yield of 55% was obtained as a greenish-black powder. Quinine sulfate (0.1 M H_2SO_4 as solvent; QY = 0.54) were chosen as standards. The QY was determined by slope method by the reference of quinine sulfate, comparing the integrated photoluminescence intensity and the absorbance value of the samples with that of the references [20]. Afterwards, the bare CDs were modified with Arg by using NHS and EDC [21]. Briefly, Arg (10 mg) was dissolved into the MES buffer (20 mL, 0.1 M, pH 5.6) with magnetic stirring, and then 23 mg NHS (10 mM) and 1.91 g EDC (50 mM) were added into the mixture to activate the carboxylic group. After 20 min, the freezing-dried CDs powder (30 mg) was added, and the mixture was stirred overnight at room temperature, subsequently, the resulting solution was purified by dialysis and Arg-CDs were obtained as a brownish solid.

The Arg-CDs were characterized by high-resolution transmission electron microscope (HRTEM; Tecnai G2 S-TWIN, FEI, USA) and dynamic light scattering (DLS; Malvern). A microplate reader (Spectra Max M5, Molecular Devices, USA) was used for fluorescence intensity measurement. The functional information of Arg-CDs was made with a Nicolet AVATAR 360 FT-IR spectrophotometer.

2.2. Cell culture

Four different cell lines (NIH 3T3, HEK 293, Hela, MCF-7) were obtained from Peking Union Medical College (Beijing, China). The cells were respectively cultured in Dulbecco's Modified Eagle Medium (DMEM, HyClone) containing 10% fetal bovine serum (FBS), 100 U/mL^{-1} penicillin and 100 mg/mL^{-1} streptomycin at 37 °C in 5% CO_2 humidified atmosphere.

2.3. Cell imaging

The inverted fluorescence microscopy (Olympus, Japan) was used to qualitatively determine the efficiency of cellular uptake. CDs or Arg-CDs (0.25 mg/mL^{-1}) from the stock solution were prepared with Dulbecco's Phosphate Buffered Saline (DPBS), and the solution (50 μL) was added to the cell media, then incubated at 37 °C in a 5% CO_2 incubator for 2 h. Before observation, the excess CDs and Arg-CDs were removed by washing 3 times with pre-warmed DPBS. Finally, the cells were observed under the fluorescence microscope and the images were taken with a digital camera.

2.4. Cell uptake study

The cells were transferred onto 24-well plates at a density of 5 × 10-4 cells well^{-1} and incubated at 37 °C, in 5% CO_2 humidified atmosphere. When the confluence of cells reached 80%, CDs and Arg-CDs were respectively added into the growth media at the final concentration of 0.12 mg/mL^{-1}. Subsequently, the growth media was collected for fluorescence detection on a Molecular Devices Spectra Max M5 microplate reader. The cells were washed with pre-warmed DPBS, and then the cellular fluorescence intensities were measured with the M5 microplate reader.

2.5. Cell viability assay

The cells were respectively seeded in a 96-well plate, at a density of 2 × 10^5 cells well^{-1} and incubated for 24 h in the incubator (37 °C, 5% CO_2), and then the culture media were replaced with 100 μL DMEM containing Arg-CDs. After incubation for 2 h, the media containing Arg-CDs was removed, and replaced with 200 mL fresh DMEM. The cell viability was measured according to the manufacturer's protocol of AlamarBlue cell viability assay reagent (Pierce). The fluorescence intensity was recorded on the microplate reader (Spectra Max M5). The wells with no CDs were used as the positive control, while the wells with no cells were considered as the blank control. The relative cell viability (mean ± SEM, $n = 3$) was expressed as [Abs (Sample − blank) / Abs (control − blank)] × 100%.

3. Results

3.1. The characteristics of Arg-CDs

The prepared Arg-CDs could freely disperse in water without extra ultrasonic dispersion, affording a transparent solution. Then, a tiny drop of the solution was placed on a cover glass, bright blue luminescence (Fig. 1A) was observed under the fluorescent microscopy at the ultraviolet excitation (330–380 nm). To further investigate the luminescence feature of Arg-CDs, the emission spectrum was recorded as the strong blue luminescence peaking around 470 nm by altering the excitation wavelengths (Fig. 1B).

The structure of Arg-CDs was uniform, monodispersed spherical particles through TEM image and the size distribution varied from 1.0 to 4.5 nm (average diameter about 2.5 nm) by TEM analysis (Fig. 1C). Furthermore, the functional groups on the surface of Arg-CDs were

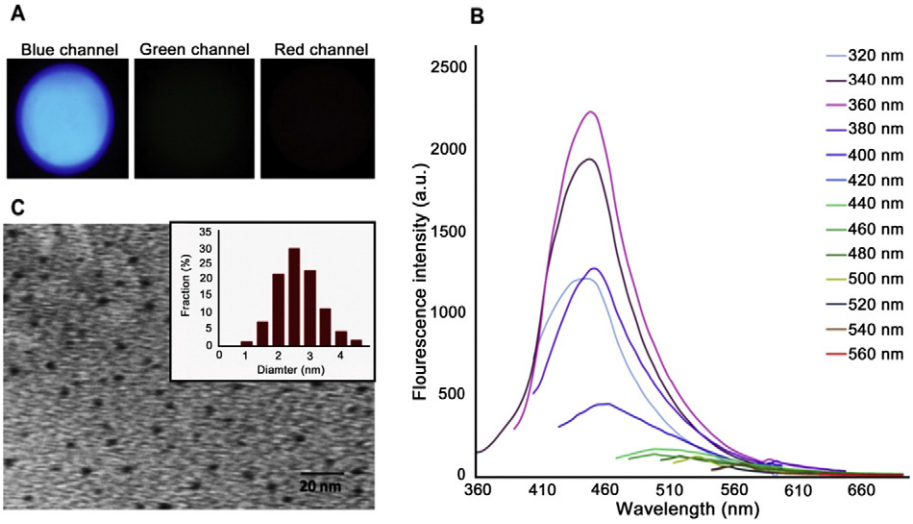

Fig. 1. The characteristics of Arg-CDs. (A) Arg-CDs aqueous solutions under ultraviolet (330–380 nm), blue (460–495 nm) and green (530–550 nm) light excitation of fluorescent microscopy; (B) Fluorescence spectra of Arg-CDs aqueous solutions under the excitation wavelengths from 360 to 700 nm; (C) TEM image of Arg-CDs. scale bar is 20 nm; the inset shows size distribution histogram.