Preparation of strong green fluorescence carbon quantum dots for cancer cell imaging

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Abstract: Carbon quantum dots (CQDs) were prepared through hydrothermal synthesis method. The TEM and FT-IR spectra showed the successful synthesis of CQDs and the well water solubility of CQDs. After CQDs were co-cultured with human breast cancer cells (MCF-7 cells), the cells were stained and emitted strong green fluorescence, indicated that CQDs had a good application prospect in cancer cell imaging.

1. Introduction
Cancer is a severe disease and kills many thousands of people every year[1]. Therefore, it is urgent to find a new thoughts for the diagnosis and treatment of cancer. Optical bioimaging is of great significance in the early diagnosis of cancer, which can accurately identify normal cells and cancer cells by using tumor biomarker[2-4]. Carbon quantum dots (CQDs) are a kind of small carbon nanoparticles, which have small size (below 10nm) and excellent optical properties and can be modified or functionalized and then combined with other groups to enhance CQDs' characteristics[5]. CQDs can be used in biological imaging because of its water solubility, low toxicity, biocompatibility and fluorescence properties[6-7]. CQDs contain a large number of carboxyl groups and hydroxyl groups. These carboxyl groups and hydroxyl groups provide a large number of reaction sites and can combine with other functional groups, so that CQDs have the potential ability to target identification of specific cancer cells[8].

Herein, novel fluorescent CQDs were prepared by water soluble nigrosine and betaine hydrochloride through one-step hydrothermal synthesis method. We characterized its microstructure and optical properties and then CQDs were combined with cancer cells to explore its cell imaging applications[9].

2. Materials and Methods

2.1 Chemicals and Materials
Water soluble nigrosine and betaine hydrochloride were purchased from Aladdin Industries Co., Ltd (Shanghai, China). Trypsin-EDTA solution, Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), Phosphate Buffered Saline (PBS), MTT Cell Proliferation and Cytotoxicity Assay Kit (MTT) were acquired from Lanbao Biotechnology Co., Ltd (Hangzhou, China). All chemicals were of analytical grade and the ultrapure water (≥ 18 MΩ·cm) water used in the experiment was self-made by purifier of Yjd RO-MB-10T (Hangzhou, China).
2.2 Synthesis of carbon quantum dots (CQDs)
Carbon quantum dots (CQDs) were prepared by one-step hydrothermal synthesis method using water soluble nigrosine and betaine hydrochloride\[9\]. 0.5g betaine hydrochloride was dissolved in 25ml ultrapure water, and then 0.25g water soluble nigrosine was added into the mixed solution and stirred until uniformity. The above uniformly mixed solution was transferred to a 50ml inner PPL liner, sealed with stainless jacket and hydrothermal synthesis was conducted at 250 °C for 7 h in a box muffle furnace. After the hydrothermal synthesis finished, the supernatant was filtered (slow-speed filter paper and 0.22 μm filter), dialyzed in the dialysis bag for 24 hours (MWCO = 1000D) and then was treated using freeze drying technology.

2.3 Cell viability test
The Cell viability of CQDs was evaluated by MTT assay. MCF-7 cells were incubated in a 96-well plates with DMEM containing 10% FBS, 1% Trypsin-EDTA at 1 \times 10^4 \text{ cells} \text{ density} to per well and incubated in an incubator overnight. Then, various different concentrations CQDs solution (0, 0.5, 1.0, 1.5, 2.0, 0.68 mg/ml, respectively) were added to each well and incubated for another 4h and 8h. Microplate reader was used to measure the absorbance at 490 nm after 20 μL MTT was added to each well.

2.4 In vitro cell imaging
MCF-7 cells were incubated in a confocal dish with DMEM containing 10% FBS, 1% Trypsin-EDTA at a density of 1 \times 10^5 \text{ cell} and incubated in an incubator overnight. 150 μL CQDs solution (0.68 mg/ml) were added to each confocal dish and cocultured for 4 hours. Confocal dish was washed 3 times with 1ml PBS and confocal images were taken by confocal laser scanning microscope.

2.5 Characterization
The CQDs microstructure and surface morphology were characterized by transmission electron microscopy JEM-2100. Fourier transform infrared (FT-IR) spectra was collected by Fourier transform infrared spectrometer Nicolet 5700. Ultraviolet visible (UV–vis) spectra were obtained by Hitachi U-3900 spectrophotometer. Fluorescence characteristics were characterized by Hitachi F-7000. Absorbance were obtained by microplate reader BioTek ELx800. Confocal laser scanning microscope images were taken by confocal laser scanning microscope FV1200-IX81.

3. Results and discussion

Figure. 1 (a) TEM image and (b) HRTEM image of carbon quantum dots (CQDs)

Figure. 1 showed the microstructure of CQDs. Figure.1 (a) showed that the shape of CQDs was spherical or quasi-spherical, uniformly dispersed, and the size of carbon dots was less than 5 nm. The particle size mainly concentrated around 2-3 nm and the average particle size was 2.69 nm. Figure.1 (b) is HRTEM image, which showed the interplanar spacing was 0.288 nm. TEM diagram proved the successful synthesis of carbon quantum dots.
FT-IR spectrum showed that there was a wide peak with strong absorption at 3450 cm\(^{-1}\), which could be attributed to the stretching vibration of O-H and N-H. 2800-3000 cm\(^{-1}\) was the characteristic absorption band of C-H. The peak 2883 cm\(^{-1}\) existed in this range indicated the existence of stretching vibration of C-H. The absorption peak at 1712 cm\(^{-1}\) could be attributed to the presence of C=O stretching vibration. C=O at 1712 cm\(^{-1}\) combined with the O-H at 3450 cm\(^{-1}\) indicated the presence of COOH. The absorption peak at 1622 cm\(^{-1}\) was attributable to C= C stretching vibration. The absorption peaks at 1217 and 1090 cm\(^{-1}\) could be attributed to the presence of C-N-C and C-O. The FT-IR spectra showed that the surface of the synthesized CQDs mainly contained water-soluble groups such as O-H and COOH so that CQDs had good water solubility.

At room temperature, the optical properties of CQDs were explored. Figure 3(a) was the UV-vis absorption spectra of CQDs. The spectra showed that there were two absorption peaks (237 nm and 260 nm) in the range of 200-300 nm, which could be attributed to the C=C bond sp\(^2\) electron \(\pi-\pi^*\) transfer and C=O bond electron \(n-\pi^*\) transfer, respectively. Absorption peak at 358 nm, which may be due to the electron \(n-\pi^*\) transfer of C-N bond and C-O bond. Figure 3(b) was the fluorescence spectra of CQDs. The spectra showed the relationship of excitation and emission wavelengths when the excitation wavelength was increased from 270nm to 320nm. It could be seen from the Figure 2(b) that the best excitation wavelength was at 280 nm, and the corresponding maximum emission wavelength was...
around 390 nm. The emission wavelength of CQDs hardly changed with the excitation wavelength changed, which might be attributed to the uniform size distribution of CQDs.

Figure. 4 The cell viability of CQDs with MCF-7 cell for 4h and 8h

The cell viability of CQDs was measured by MTT assay. It could be seen from Figure. 4 that the cell viability value was higher than 85%, which was over than the standard value of 70% according to the ISO 10993-5:2009[10]. This proved that the CQDs had a very low cytotoxic. CQDs and MCF-7 cells were co-cultivated for 4h or 8h and the cell viability value was very high and the gap was small, which proved that CQDs had good cell cytocompatibility and could be potential used in practical biomedical applications. The cell viability results showed that CQDs had the potential application in vitro imaging diagnosis of cancer.

Figure. 5 Confocal laser scanning microscope images of MCF-7 cells after incubation with carbon quantum dots (left column: merged images, middle column: dark field image excited with a 488 nm laser right column: bright field image)

Figure. 5 was a confocal laser scanning microscope images taken after co-cultivate of MCF-7 cells and CQDs for 4 hours. The bright field on the right showed that the cell morphologies were normal and complete without being broken, which also proved that CQDs had good cytocompatibility, low toxicity, and minor impact on cancer cells. MCF-7 cells were excited by 488 nm laser, images showed that the cells had uptaken CQDs and be stained, emitted green fluorescence. Therefore, we could expect that CQDs have great application potential in cancer cell imaging.
4. Conclusions
In this work, we synthesized carbon quantum dots (CQDs) by using water soluble nigrosine and betaine hydrochloride through hydrothermal synthesis method. CQDs had good water solubility and cell compatibility. MCF-7 cells uptook CQDs and emitted green fluorescence. In addition, CQDs had very low cytotoxicity and small damage to cells with time going by. Therefore, CQDs may be possible combine with other targeted substances to realize the exact recognition of cancer cells, which provide potential feasibility of cancer optical bioimaging diagnosis.

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