Colloids and Surfaces B: Biointerfaces 171 (2018) 241–249

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The effect of surface charge on the cytotoxicity and uptake of carbon quantum dots in human umbilical cord derived mesenchymal stem cells

Junyan Yan[a,i,1], Shulan Hou[a,g,1], Yanzhen Yu[a,h], Yong Qiao[a], Tongqian Xiao[a,f], Yan Mei[d], Zhijun Zhang[a], Bin Wang[e], Chih-Ching Huang[b], Chia-Hua Lin[c,*], Guangli Suo[a,*]

[a] CAS Key Laboratory of Nano-Bio Interface, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou, Jiangsu, 215123, China
[b] Department of Bioscience and Biotechnology, National Taiwan Ocean University, Keelung, 20224, Taiwan
[c] Department of Biotechnology, National Formosa University, Yunlin, 63208, Taiwan
[d] Greepharma Inc., Nanjing, Jiangsu, 211100, China
[e] Center for Clinic Stem Cell Research, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008, China
[f] University of Chinese Academy of Sciences, Beijing, 100049, China
[g] School of Pharmacy, Xi'an Jiaotong University, Xi'an, Shannxi, 710061, China
[h] University of Science and Technology of China, Hefei, Anhui, 230026, China
[i] School of Life Science, Shaoxing University, Zhejiang, 312000, China

ARTICLE INFO

Keywords:
Carbon quantum dots
Human umbilical cord derived mesenchymal stem cells
Cytotoxicity
Surface charge
Labeling efficiency

ABSTRACT

Carbon quantum dots (CQDs) are emerging as an ideal agent for efficient stem cell labeling. In current study, we synthesized a series of CQDs carrying different surface charges by changing the mass ratio of diammonium citrate (DC) and spermidine (Spd), and evaluated the effects of different surface charges on the cytotoxicity, cellular uptake, stability in human umbilical cord derived mesenchymal stem cells (hUCMSCs). We ascertained the optimal labeling time (24 h) and subtoxic concentration (50 μg/mL) of all different charged CQDs. Our results demonstrated that, although positively charged CQDs are more cytotoxic and have lower photoluminescence (PL) compared to negative CQDs, they still have higher labeling efficiency for their higher uptake capacity. We found that relatively weak positive surface charges enabled CQDs to possess good biocompatibility and labeling efficiency in hUCMSCs. This work will helpfully contribute to the design and optimization of CQDs for tracking stem cells and further benefit to clinical research and application.

1. Background

Mesenchymal stem cell (MSC), one kind of multipotent stem cell, has generated a great deal of excitement as a potential source of cell-based therapies [1–5]. After transplantation in the host organism, it is needed to monitor the fate of transplanted cells, including their distribution, survival, differentiation, and longevity over time [6–9]. Quantum dots (QDs) were usually composed by groups of II-VI or III-V elements, and their sizes ranged from 2 to 10 nm [10]. Compared with traditional fluorophores such as GFP, FITC and rhodamine, QDs possess many advantages due to their optical properties for cellular label and imaging, such as remarkable resistance photo bleaching, high fluorescence efficiency, size-tunable emission, broader absorption spectra and narrower emission spectra [11]. Compared with luciferase used for cell imaging, QDs avoid cellular aberrance potentially caused by integration of exogenous luciferase gene and the side effect of substrate (luciferin) on cells.

The carbon based QDs (CQDs) were regarded as promising probes for *in vitro* and *in vivo* imaging applications owing to their superior optical properties over conventional QDs, such as relatively high chemical and optical stabilities, superior water dispersibility and excellent biocompatibility [12–14]. However, some studies reported that different synthetic methods, particle sizes, and surface chemistry modifications affected the biological toxicities and uptake of CQDs [15–17]. So far, CQDs potential cytotoxicity and instability in biological environment are still ambiguous.

The effects of surface charge on uptake and biological function of different nanoparticles in MSCs have been widely studied. One study reported that positively charged mesoporous silica nanoparticles (MSNs) were quite biocompatible in 3T3-L1 cells and human MSCs (hMSCs); the uptake of MSNs by hMSCs could be regulated by a threshold of positive surface charge and the modulation of surface

* Corresponding authors.
E-mail addresses: vicchlin@nfu.edu.tw (C.-H. Lin), glsuo2013@sinano.ac.cn (G. Suo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.colsurfb.2018.07.034
Received 18 April 2018; Received in revised form 22 June 2018; Accepted 16 July 2018
Available online 17 July 2018

charge on the uptake was specific to cell type [18]. Chen et al. reported that positively charged hydroxyapatite (HAP) nanoparticles showed stronger improvement for osteoblast cell viability and cell proliferation [19]. Li et al. demonstrated that gold nanoparticles (AuNPs) with different surface charges induced differential cell response on MSCs osteogenesis [20]. In their study, positively charged AuNPs-NH_3 showed higher cellular uptake and less effect on alkaline phosphatase (ALP) activity and matrix mineralization in hMSCs compared to negatively charged AuNPs-COOH. Nevertheless, the effects of surface charge on cytotoxicity and uptake of CQDs in hMSCs have not been studied in depth.

In this study, based on one-step carbonization method [21], we developed a novel straightforward method to synthesize CQDs carrying different surface charges by changing the mass ratios of diammonium citrate (DC) and spermidine (Spd). Furthermore, we investigated the effects of different surface charges on CQDs cell-uptake and cytotoxicity, and on proliferation, stemness, self-renewal and differentiation capacity of human umbilical cord derived mesenchymal stem cells (hUCMSCs). Our data suggested that relatively weak positive surface charges enabled CQDs to strike good balance between biocompatibility and labeling efficiency in hUCMSCs. This study provides helpful insight into the design of CQDs for tracking stem cells and further benefit to clinical research and application of CQDs.

2. Methods

2.1. Synthesis and characterization of CQDs

CQDs were synthesized using a modified bottom-up carbonization method based on one previously reported [21]. Like Table S1, different mass ratios (1:0, 1:0.25, 1:1, 1:2.5) of diammonium citrate (DC) and spermidine (Spd) were mixed and ground with an agate mortar and pestle for 5 min. Then, the mixtures were moved in beaker and heated at 180 °C for 2 h in a heating furnace. After cooling down to room temperature, the residues were dissolved in 10 mL water and sonicated for 1 h. After centrifugation by 35,000 *g* for 30 min at 4 °C, the collected supernatants were dialyzed against deionized water through a dialysis membrane (MWCO = 1.0 kD) for 5 h. Subsequently, the purified supernatants were lyophilized and dissolved in water. Thereto, four different CQD samples synthesized with different mass ratios of DC and Spd were obtained and named by C-a, C-b, C-c and C-d. The CQDs were put into 4 °C refrigerator for long-term storage. Here, Spd alone was also considered as one source of carbon and used to synthetize CQD following the same process. Transmission electron microscopy (TEM) images of the CQDs were acquired using Tecnai G2 F20 S-Twin TEM (FEI, USA). The zeta potentials of CQDs in water solution were measured using Zetasize (ZEN3600; Malvern Instruments, UK). Ultraviolet-visible (UV–Vis) absorption spectra of CQDs solutions were recorded using monochromatic microplate spectrophotometer (Synergy 4 Multi-Mode; Biotek Instruments, USA). The photoluminescence (PL) spectra of CQDs dispersion were measured by multilabel counter ARVO mx 1420 (ParkinElmer, MA).

2.2. Source and culture of hUCMSCs

The hUCMSCs were gifted and the cells qualification was identified by Nanjing Gulou Hospital. The study was approved by Local Ethics Committee, and all participants were given informed consent to participate. Cells were cultured in basal Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin-streptomycin (Beyotime Institute of Biotechnology, Jiangsu, China) in a humidified atmosphere of 5% CO_2 in air at 37 °C. The hUCMSCs within passage 8 were used in all experiments.

2.3. Cell viability assay

Cell viability was assessed using a cell counting kit-8 (CCK8, Beyotime, China). Briefly, cells were co-cultured with different concentrations of CQDs in 96-well plates for 72 h at a density of 5×10^3 cells/well. CCK8 reagent (10 μL) was then added to each well and incubated for another 4 h before measurement. The absorbance at 450 nm was measured using Multilabel Reader (PerkinElmer, Singapore). The relative cell viability was determined by absorbance values of cells treated with CQDs normalized to those of cells without CQDs treatment control.

2.4. Reactive oxygen species (ROS) assay

Intracellular ROS were detected using the oxidant-sensitive dye carboxy-H_2DCFDA (Invitrogen). Cells were plated at a density of 5×10^3 cells/well in 96-well plates and cultured for 24 h, and then co-cultured with CQDs (50 and 100 μg/mL) for the other 24 h. After the incubation, the cells were added with 100 μ L of 100 μM carboxy-H_2DCFDA, and cultured for 30 min at 37 °C. The fluorescence intensity in each well was measured at an excitation of 485 nm and an emission of 585 nm. To eliminate the effect of fluorescence of CQDs itself on DCF fluorescence detection, the cells labeled with different types of CQDs and without adding carboxy-H_2DCFDA were used as zero setting controls for each type of CQDs treatment.

2.5. Fluorescence microscopy

The hUCMSCs were plated in Confocal culture dish and culture for overnight, and then co-cultured with CQDs (50 μg/mL) for 24 h. After incubation, cells were washed twice with PBS to remove unlabeled CQDs and fixed with fixing solution (Beyotime, China). The fluorescence images of hUCMSCs labeled by CQDs were acquired by Confocal laser scanning microscopy (Nikon A1, Japan). The fluorescent intensity of CQDs in the cells was analyzed using ImageJ software.

2.6. Uptake of CQDs by hUCMSCs

To assay uptake rate of CQDs affected by varying incubation times, hUCMSCs were plated at a density of 5×10^4 cells per well in 12-well plates and then co-cultured with 50 μg/mL of CQDs for 0, 3, 6, 9, 12, 24, 48 and 72 h. To assay uptake rate affected by varying CQDs concentrations, hUCMSCs were plated at a density of 5×10^4 cells per well in 12-well plates and then co-cultured with different concentrations of CQDs (0, 50, 100, 200, and 400 μg/mL) for 24 h. After co-cultured with CQDs, cells were harvested and washed twice with PBS, and then analyzed by flow cytometry (BD Accuri C6). The FITC fluorescence channel (FL-1) was used to detect cellular uptake of CQDs. The hUCMSCs without CQDs treatment were used as no detectable control for gating the CQDs labeled cells. Following the method described before [22], labeling efficiency was represented as the percentage of detectable cells by flow cytometry in FITC fluorescence channel from total 5000 tested cells. Labeling efficiencies of different types of CQDs (50 μg/mL) in hUCMSCs were analyzed and compared after 24 h of co-culture. The retention efficiency of each type of CQDs in hUCMSCs was determined by the ratio of labeling efficiency to corresponding photoluminescence (PL) intensity.

2.7. Stability of CQDs in hUCMSCs

To label the hUCMSCs with CQDs, 2×10^5 cells were plated in one well of 6-well plates and co-cultured with CQDs (50 μg/mL) for 24 h. After labeling, cells proliferation and fluorescence intensity of CQDs in cells were measured at different time points from 0 to 12 days. At each time point, cells were washed twice with PBS to remove unlabeled CQDs and collected by trypsinization. The fluorescent intensity of cells