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Cytotoxicity effect assessment of acid purified carbon nanotubes modified with cetyltrimethyl ammonium bromide

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Abstract: The cytotoxicities of single-walled carbon nanotubes (SWNTs) and acid purified single-walled carbon nanotubes (SWNT-COOH) were investigated by spectroscopic analysis. Cell viability and cell apoptosis were applied to assessing the cytotoxicity of SWNT-COOH, cetyltrimethyl ammonium bromide (CTAB) and acid purified carbon nanotubes modified with cetyltrimethyl ammonium bromide (SWNT-COOH/CTAB). The results indicate that SWNTs are more toxic than SWNT-COOH. Concentration and time−curve analyses indicate that cytotoxicity of SWNT-COOH/CTAB is more related to the toxicity of the surfactant CTAB. The cytotoxicity effect of CTAB and SWNT-COOH/CTAB is acceptable at low concentrations (0.5−25 μg/mL). The cytotoxicity observation suggests that SWNT-COOH/CTAB can safely applied to biomedical field at low concentrations (0.5−25 μg/mL).

Key words: single-walled carbon nanotube; cetyltrimethyl ammonium bromide; cytotoxicity; acid purification; apoptosis

1 Introduction

The potential use of single-walled carbon nanotubes (SWNTs) in scientific research was first published in 1993 [1]. Owing to the unique optional, thermal, chemical and mechanical properties of carbon nanotubes, they gained and triggered significant interest in the engineering field [2,3]. Then they attracted great attention for various biomedical applications [4], such as imaging, biosensors, drugs, biological scaffold, gene delivery and cancer therapy [5,6]. But their cytotoxicity effect is still a controversial issue which restricts its biomedical applications [7]. There are many factors such as dispersibility, impurities, length and surfactants contribute to the cytotoxicity of CNTs [8,9]. The carbon nanotubes (CNTs) generate oxidative stress, decrease cell viability and induce apoptosis [10]. So, various methods are tried to decrease its cytotoxicity. Carbon nanotubes purified by carboxylic acid (CNT-COOH) have been widely used because they could well reduce impurities of catalysts, shorten the nanotubes and make some surfactants easier to conjugates with them. But ZHU et al [11] found that CNTs-COOH were more toxic than CNTs, as they reduced the cell viability in a dose-dependent manner. So, the cytotoxicity of acid purified single-walled carbon nanotubes (SWNTs-COOH) is still a controversial issue [12]. To date, only a few studies have compared the cytotoxicity of SWNTs and SWNTs-COOH [11,12].

Various surfactants were used to disperse carbon nanotubes [13]. Surfactants modified to CNTs could decrease its cytotoxicity and get it more easily combined with other molecules. CTAB is a cationic and non-covalent surfactant, which has been widely applied as a wally surfactant in electrode and biological biosensor [14,15]. In our previous study, it was found that CTAB has a stronger tendency to bind with nucleic acid [16], so it maybe has a bright future to be used as a surfactant conjugated to single-walled carbon nanotubes (SWNT-COOH/CTAB) for the purpose of gene transfection. ZHANG et al [17] reported that CTAB-PBCA

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(cetyltrimethyl ammonium bromide polybutylcyano-acrylate) nanoparticles were avirulent to $HepG_2$ cells. While GUO et al [18] found that modification with CTAB caused a damage to cells. So before considering its biological applications, cytotoxicity effect on it should be taken into consideration. CTAB can easily bind with negatively charged nucleic acid to make single-walled carbon nanotubes gene carrier. So it is meaningful to assess the cytotoxicity with CTAB, a cationic surfactant. In our study, the cytotoxicity and apoptosis effect of SWNTs, SWNT-COOH and SWNT-COOH/CTAB were evaluated.

2 Experimental

2.1 Preparation of SWNT conjugates

In this experiment, SWNTs (out diameter 1−2 nm, length 1−3 μm) and SWNT-COOH (out diameter 1−2 nm, length 1−3 μm) powders were purchased from Chengdu organic chemical company (Sichuan, China). CTAB powders were obtained from State Key Laboratory for Powder Metallurgy, Central South University, China.

The SWNTs, SWNTs-COOH and SWNT-COOH/CTAB solution were prepared by ultrasonic treatment and centrifugation method. First, SWNT powder, SWNT-COOH powder, mixture of SWNT-COOH and CTAB powder were dispersed in deionized water using ultrasonic tip (MODEL:CV188, China) operating at 70 W for 120 min to obtain SWNT suspension, SWNT-COOH suspension and SWNT-COOH /CTAB suspension. Dispersion of SWNTs, SWNT-COOH and SWNT-COOH/CTAB suspension were prepared at concentrations ranging from 0.5 to 100 μg/mL. In this process, concentration of the surfactant was kept constant (CTAB 0.3 mg/mL). Then, centrifugation (eppendorf centrifuge 5417R, Germany) with SWNT suspension, SWNT-COOH suspension and SWNT-COOH/CTAB suspension were respectively conducted at 13000*g* for 30 min. Then, we discarded the precipitates containing catalysts, bundles of nanotubes and amorphous carbon debris.

2.2 Characterization of water-dispersible SWNTs

SWNTs and SWNT-COOH powders were determined qualitatively by X-ray diffraction (XRD). Meanwhile, their quantitative analyses were performed through thermogravimetric analysis (TGA) and energy disperse spectroscopy (EDS). Thermogravimetric analysis was performed on SWNTs and SWNTs-COOH. The heating rate was 10 °C/min. The temperature was increased from room temperature to 700 °C in the presence of normal atmosphere.

2.3 Cell culture

HeLa cells obtained from Institute of Biochemistry and Cell biology(China) were used for evaluating toxicity. Cells were grown in a humidified incubator at 37 °C (5%CO_2) using DMEM medium (Gibco), which was supplemented with 10% fetal bovine serum (Gibco).

2.4 Analysis of cell viability

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay(Sigma) was used to determine cell viability. The metabolic activity of cells is proportional to the color density formed. The cells viability was calculated through the formula $(OD_e/OD_c)\times100\%$, OD_e represents the absorbance value of experimental group, and OD_c represents the absorbance value of control group).

HeLa cells were grown in 96 well microplates for 12 h. These cells were washed with phosphate buffered saline (PBS), and the medium was changed to 10% fetal bovine serum (FBS). Then, these cells were individually exposed to SWNT-COOH, surfactant CTAB, and CTAB coated SWNT-COOH suspensions, with the concentration varying in the range of 0.5−100 μg/mL for 48 h. For another group, CTAB coated SWNT-COOH suspensions were added into HeLa cells with the concentration varying in the range of 0.5−100 μg/mL. Then they were cultured for 24, 48, and 72 h. HeLa cells in the culture medium devoid of these materials were used as the control. After their incubation, 15 μL of MTT stock solution (5 mg/mL) was added to each well, then the plates were further incubated at 37 °C for 4 h in 5% CO_2 humidified incubator. This resulted in the formation of purple-colored formazan crystals in the living cells, then the supernatant was removed gently, and the purple products were lysed with 150 μL dimethyl sulfoxide (DMSO). The plate was homogeneously agitated for 10 min on a shaker. Then, the absorbance was measured at a wavelength of 490 nm using a microplate reader. Cell viability was expressed as a percent of the control group.

2.5 Observation of nuclear morphology

Nuclear morphology of HeLa cells was examined via fluorescence microscope (OLYMPUS ZX71 TH4-200, Japan) after staining the cells with fluorescein dye: 33342 kit (Beyotime Jiangsu, China). HeLa cells were incubated in 24 well plates using media containing different concentrations of SWNT-COOH/CTAB at 37 °C for 48 h. After thoroughly washing them twice with PBS, the buffer supplied in the kit was added to each well. The cells were stained with Hochest33342 (5 μg/uL) at 4 °C for 20 min and kept in dark. Then, the cells were observed by a fluorescence microscope (OLYMPUS ZX71 TH4−200, Japan). Apoptotic cells were identified through the nuclear morphology changes.