Carbon 108 (2016) 529–540

Contents lists available at ScienceDirect

Carbon

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

Diameter size and aspect ratio as critical determinants of uptake, stress response, global metabolomics and epigenetic alterations in multi-wall carbon nanotubes

Nivedita Chatterjee [a], Jisu Yang [a], Suhkmann Kim [b], Sang Woo Joo [c], Jinhee Choi [a, *]

[a] School of Environmental Engineering, University of Seoul, 163 Siripdaero, Dongdaemun-gu, Seoul, 130-743, South Korea
[b] Department of Chemistry, Pusan National University, Busan, 609-735, South Korea
[c] Department of Chemistry, Soongsil University, Seoul, 156-743, South Korea

ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form
7 July 2016
Accepted 15 July 2016
Available online 18 July 2016

ABSTRACT

The present study aimed to refurbish the fact of size and aspect ratio specific biological response of MWCNT in different cell systems. The bioactivities of three MWCNTs, differing in length/diameter [10–30 μm/20–30 nm (LW); 10–30 μm/8–15 nm (LN); 0.5–2.0 μm/8–15 nm (SN)], were then evaluated in terms of uptake and various toxicological end points (anti-proliferative, oxidative stress, inflammatory, DNA damage-repair and differential mode of marker gene expressions) in two different cell types - human bronchial epithelial (BEAS-2B) and human hepatoma (HepG2) cell lines. The surface enhanced Raman scattering (SERS) with dark-field microscopy attested the intracellular uptake of SN, but not the LW/LN, supports length dependency. The MWCNTs showed size specific stress responses (greatest in short sized one, SN) in both type of cells and aspect ratio dependent pro-fibrogenic potentiality, the LN with highest aspect ratio exhibited highest fibrogenicity in lung cells. Furthermore, alterations in global metabolomics and global DNA methylation status (DNMT3B dependent hypo-methylation in BEAS-2B cells and hyper-methylation in HepG2 cells) were also evident. Taken together, our findings provide evidence of variations in stress response, epigenetic modifications and global metabolomics alterations in association with differential size and aspect ratio of MWCNT which might be translated to the safe-by-design concept as well as risk assessment.

1. Introduction

Multiwalled carbon nanotubes (MWCNTs) are concentrically stacked cylindrical tubes of graphitic carbon and possess unique physical, thermal and electrical properties, including their high surface area, high mechanical strength, ultra-light weight, enhanced electrical conductivity, and excellent physical-chemical-thermal stability, have led to wide range of applications not only in the field of electronics, emission devices, energy storage devices but also emerged as drug delivery carriers, biosensor, therapeutics, diagnostic materials in the biomedical and nanomedicine field [1–4]. Nevertheless, due to their extremely hydrophobic nature MWCNTs possess less dispersibility and can aggregate in aqueous solution. Various studies including a recent review documented that the surface modifications with various chemical groups enhance the dispersion and biocompatibility of MWCNTs and thus functionalized CNTs rise as promising novel materials for a variety of biomedical applications [5]. The functionalized MWCNTs open up enormous potentiality of applications, specifically in diagnostic and therapeutic nanomedicine, such as, in ultrasonography [6], as immunomodulator systems [7,8]. Among the main chemical modifications, hydroxylation is one of the most effective one, since the hydroxyl group could conjugate with other functional molecules, such as binding with hemoglobin, gamma globulin and transferrin for greater biocompatibility of MWCNT [9,10]. Rapid growth and applications of MWCNTs, including the functionalized one, raises the concerns about the potential risks and toxicities for public health, environment, and workers associated with the manufacture [11]. Although numerous foregoing studies related to the biological response and toxicities of MWCNTs at the molecular, cellular, organisms' levels have been published, the results are often

Abbreviations: MWCNT, multi-walled carbon nanotubes; LW, long length and wide diameter MWCNT (Length: 10–30 μm, diameter: 20–30 nm); LN, long length and narrow diameter MWCNT (Length: 10–30 μm, diameter: 8–15 nm); SN, short length and narrow diameter MWCNT (Length: 0.5–2.0 μm, diameter: 8–15 nm).

* Corresponding author.
E-mail address: jinhchoi@uos.ac.kr (J. Choi).

http://dx.doi.org/10.1016/j.carbon.2016.07.031
0008-6223/

conflicting. The nano-bio interactions of MWCNTs vary according to the length, diameter, surface functionalization, aspect ratio of the MWCNT as well as the target cell types [12–18]. At present, it is still ambiguous that which parameter play the most important role in MWCNTs induced toxicological effects. Therefore, the adoptions of safe-by-design concept as well as appropriate exposure controls are critical for the successful introduction of MWCNT-based products for future applications.

Here, we hypothesized that differential size and aspect ratio, neither the length nor diameter alone, influence the biocompatibilities of functionalized MWCNTs. To investigate the size and aspect ratio dependent effects three different types of hydroxylated MWCNTs, differing in length (L) and outside diameter (D), [L:10–30 μm, D: 20–30 nm (LL); L:10–30 μm, D: 8–15 nm (LS); L: 0.5–2.0 μm, D:8–15 nm (SS)], were chosen. To accomplish the cell specific biological response, we used two cell culture systems, human bronchial epithelial cells (BEAS-2B cells) and human hepatoma cells (HepG2 cells), as model systems. The BEAS-2B cells were chosen as lungs represent the main potential target organ during manufacture and processing of nanomaterials (inhalation toxicity model) and the HepG2 cells were selected by considering MWCNT as of potential drug delivery carriers and possible accumulation in the liver system (hepatotoxicity model). Besides the physico-chemical characterizations of all MWCNTs, the comparative nano-bio interactions were evaluated with uptake, cytotoxicity, oxidative stress, inflammatory and profibrogenic response, DNA damage-repair, global metabolomics changes, alterations in global DNA methylation (with the expression of DNA methylation machinery as well) status and the marker gene expressions related to each endpoint.

2. Experimental section

2.1. Characterization of MWCNT

The commercial hydroxylated MWCNT differing in their length and diameter [long length-wide diameter (LW), long length-narrow diameter (LN) and short length-narrow diameter (SN)] were purchased from the CheapTubes.com (Cambridgeport, VT, USA) and were additionally characterized, further, with X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM) and dynamic light scattering spectrometer (DLS).

XPS (Sigma Probe, ThermoVG, UK) was used to examine the surface chemical modification of the MWCNT. The data were obtained by a hemispherical analyzer equipped by a monochromatic Al X-ray sources (15 kV, 100 W, 400 μm) operating at a vacuum (2×10^{-9} mb). The XPS peaks were analyzed by using Gaussian components after a Shirley background subtraction. The O/C atomic ratios of the samples were obtained by using peak area ratios of the XPS core levels and the sensitivity factor (SF) of each element in XPS.

Structure and shape were investigated by TEM (Carl Zeiss LIBRA 120). The samples for TEM were prepared by drop casting a diluted suspension (50 mg/L) onto a carbon film with 300 square mesh copper grids and dried at room temperature for 24 h.

The size distribution and ζ-potential of the MWCNT (20 mg/L in DMEM culture media) were evaluated by using a photal dynamic light scattering spectrometer (DLS) (ELSZ-1000, Otsuka Electronics Co. Inc.).

2.2. Cell culture and treatment with MWCNT and inhibitors

BEAS-2B cells (human bronchial epithelial cells) were cultured in DMEM/F12 (GIBCO) and HepG2 cells (human liver carcinoma cells) were cultured in MEM (GIBCO) and both were supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) antibiotics, at 37 °C a 5% CO_2 atmosphere.

The MWCNT was freshly prepared in cell culture medium (DMEM/F12 or MEM) at the desired concentrations with appropriate amount from the stock (1000 mg/L in distilled water) and was sonicated for 15 min before biological exposure.

Blocking of uptake using endocytosis inhibitors and blocking of ROS formation using N-acetylcystine (NAC), trolox and diphenylene iodonium (DPI) as was described previously [19].

2.3. Cytotoxicity and cell viability assessment

Cytotoxicity of each MWCNT were determined by EZ-Cytox cell viability assay kit (Daeil Lab Service, Korea) based on the cleavage of the tetrazolium salt to water-soluble formazan by succinate-tetrazolium reductase as well as standard trypan blue (Invitrogen, USA) exclusion method as was described previously [19]. Three separate experiments were performed for all concentrations in triplicate, and ECs (EC_{10}, EC_{20}, EC_{50} and EC_{90}) were calculated by using four parametric logistic equations.

2.4. Colony formation and morphology changes

The Colony formation assays were carried out at 5 mg/L for 10 days in BEAS-2B cells and 15 days in HepG2 cells as described previously [20].

2.5. Cellular uptake analysis by surface enhanced Raman scattering (SERS) combined with dark-field microscopy (DFM)

The cells were plated on poly-L-lycine coated cover glass (at 1×10^5 cells/ml) and exposed to MWCNT at 1 mg/L for 24 h. The cellular uptake behaviors of MWCNT were detected by using the technique of surface enhanced Raman scattering (SERS) combined with dark-field microscopy (DFM) as was described by Park et al. [21]. Briefly, cellular uptakes of MWCNT were monitored using DFM with a Leica DMLM upright microscope and a high-resolution CytoViva 150 adapter. Raman spectra were obtained by use of a Raman confocal system model 1000 spectrometer (Renishaw) equipped with an integral microscope (Leica DMLM). Spontaneous Raman scattering was detected with 180° geometry by use of a Peltier cooled (−70 °C) CCD camera (400 × 600 pixels). An appropriate holographic supernotch filter was set in the spectrometer for 632.8 nm from a 20 mW aircooled HeNe laser (Melles Griots Model 25 LHP 928) with the plasma line rejection filter. The infrared spectra were obtained using a thermoelectron 6700 Fourier-transform infrared spectrometer with a nominal resolution of 4 cm^{-1} and 256 scans.

2.6. Total RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from MWCNT treated samples (respective EC_{20} for 24 h) were extracted by using RNA extraction kit (NucleoSpin, Machereye-Nagel) and the quantity and quality of RNAs were detected in spectrophotometer as well as with agarose gel separation. Synthesis of cDNAs was performed by a reverse transcriptase (RT) reaction and the PCR amplification was carried out as was described previously [19]. The primers used in this study were listed in Supplementary materials (Table S1). The gene expressions were normalized by using GAPDH and ACTB as a housekeeping genes.

2.7. Image analysis

The intracellular ROS were measured with DCFDA (25 μM,