Chemosphere 214 (2019) 130–135

Contents lists available at ScienceDirect

Chemosphere

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

Short Communication

Candle soot derived carbon nanoparticles: Assessment of physico-chemical properties, cytotoxicity and genotoxicity

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HIGHLIGHTS

- Physico-chemical properties, cytotoxicity and genotoxicity of CNPs was carried out.
- CNPs have insignificant cytotoxicity and genotoxicity.
- No significant DNA damage is observed in CNPs exposed V-79 cells.
- Could be used as an alternative of expensive carbon materials.

ARTICLE INFO

Article history:
Received 4 July 2018
Received in revised form
17 September 2018
Accepted 18 September 2018
Available online 19 September 2018

Handling Editor: Willie Peijnenburg

Keywords:
Carbon nanoparticles
Candle soot
Cytotoxicity
Reactive oxygen species assay
Genotoxicity

ABSTRACT

In this study, an evaluation of physico-chemical properties, cytotoxicity and genotoxicity of candle soot derived carbon nanoparticles (CNPs) was carried out. Several physico-chemical characterizations including scanning electron microscopy, transmission electron microscope, Brunauer-Emmet-Teller surface area and pore-size distribution, X-ray diffraction, Fourier transform infrared and Raman spectroscopy were implemented to characterize prepared CNPs. Propidium iodide uptake, reactive oxygen species assay and trypan blue exclusion and comet assay tests were executed to determine the toxicity of CNPs. It is found that the CNPs have insignificant cytotoxicity and genotoxicity and could be used in diverse biological and environmental applications as an alternative to expensive less toxic carbon materials.

A number of carbon nanomaterials including carbon nanotube (CNT), carbon nanofiber, graphene, fullerene, graphene/graphite nanosheets, and carbon nanorod/nanoions have been systematically used in the nanotechnology field for several environmental and biological applications (Ashfaq et al., 2013; Singh et al., 2013, 2014; Khare et al., 2018; Tripathi et al., 2018). These carbon nanomaterials demonstrate excellent optical, electrochemical, electrical, biocompatible, thermal, mechanical and adsorption characteristics along with drug delivery vehicles, which facilitate their use in diversified fields (Ashfaq et al., 2017; Coleman et al., 2017; Li et al., 2017; Tripathi et al., 2017a, 2017b; Pankaj et al., 2018). Nevertheless, among all, sp^2 hybridized graphitic carbon are considered more favourable for different kind of applications (Singh et al., 2018). Recently, carbon nanoparticles (CNPs) are being implemented for various end applications due to several aforementioned features. Different approaches have been applied to synthesise CNPs including laser ablation, chemical oxidation, electrochemical oxidation, combustion/hydrothermal, supported synthesis and microwave heating (Zhou et al., 2012; Kakunuri and Sharma, 2015; Tripathi et al., 2017c; Xu et al., 2017). Among all, candle soot (CS) derived CNPs are simple, easy to synthesise and inexpensive. Nonetheless, it is assumed that graphitic carbon nanomaterials are

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https://doi.org/10.1016/j.chemosphere.2018.09.112
0045-6535/

less toxic among all nanomaterials but silently show cytotoxicity. Even, single walled CNTs reveal less toxicity as compared to multi-walled CNTs. Consequently, there is need to investigate individual cytotoxicity of these carbon nanomaterials because they are fabricated from different methodology, source, having different shape and size along with different physico-chemical properties. The present study emphasises on physico-chemical, cytotoxic and genotoxic properties of CS derived CNPs. CS derived CNPs are considered as relatively novel carbon nanomaterials among the existed carbon members. Several cytotoxicity observations have been revealed for different carbon materials (Li et al., 2010; Singhal et al., 2015). However, as per authors' belief, the physico-chemical, cytotoxic and genotoxic assessment are made first time collectively in this study.

CS was collected in an inverted glass beaker from the tip of locally procured candle (diameter = 20 mm). Milli-Q water was used as solvent for making required solutions in this study. All the chemicals used in this study were of high purity. In brief, nitric acid (69%) and absolute ethanol were procured from Merck, India. Dulbecco's modified eagle's medium (DMEM), Fetal bovine serum (FBS), trypsin (0.25%, trypsin-EDTA-1X), antibiotic/antimycotic solution (10000 U/mL Penicillin, 10 mg/mL Streptomycin and 25 mg/mL Amphotericin B), phosphate buffer saline (PBS, Ca (II), Mg (II) free with pH = 7.4) were obtained from Life Technologies (Invitrogen bioservices) Pvt. Ltd, India. Sodium bicarbonate ($NaHCO_3$), propidium iodide and trypan blue solution (0.4%) were purchased from Sigma-Aldrich, India.

It is believed that CNPs contain >90% carbon element (Liu et al., 2007). The CNPs were partial hydrophobic and less soluble in common solvent when collected. Thereafter, CNPs were treated with 8 cycles of each 10 M nitric acid and ethyl alcohol for removal of any existed impurity. After treatment, these CNPs become soluble in water and ethanol. Furthermore, precipitate of CNPs were washed with Milli-Q water several times until pH becomes 7.0 using vortex (Vibramax 110, Heidolph, Germany) and centrifuged at 8000 rpm (centrifuge 5804, Eppendorf, Germany) followed by vacuum oven drying at 150 °C for 8 h. After drying glittering CNPs were obtained and demonstrate great dispersibility in aqueous medium. These treated CNPs were used for determining different characterizations including scanning electron microscopy (SEM, Quanta 450 FEG, FEI, The Netherlands), transmission electron microscope (TEM, Tecnai TM G2 Sprit, FEI, The Netherlands), Brunauer-Emmet-Teller surface area (S_{BET}) and pore-size distribution (PSD, Autosorb-1C, Quantachrome, USA), X-ray diffraction (XRD, X'Pert^3; Powder, PAN analytical, The Netherlands, $\lambda = 1.542$ Å), Fourier transform infrared (FT-IR, Tensor 27, Bruker, Germany), Raman analysis (Alpha, Witec, Germany, using the Ar-ion laser, $\lambda = 532$ nm with CCD detector) and Zeta potential using zeta sizer nano series (Nano ZS, Malvern Instruments Ltd, United Kingdom). Chinese hamster lung fibroblast (V-79) cells were procured from American Type Culture Collection (ATCC) USA and maintained in DMEM supplemented with 10% FBS, 0.2% $NaHCO_3$, 1% antibiotic and antimycotic solution (10 m$props_value{literPattern}/L) at 37 °C in humidified incubator with 5% CO_2 and 95% air. At 80–90% confluency, cells were harvested using 0.25% trypsin-EDTA solution and were seeded into 96 wells plate, 12 wells plate and 6 wells plate according to the requirement of experiments (1×10^5 cells/mL). Prior to treatment, cells were allowed to attach the culture surface for 18 h. CNPs suspension were prepared in above medium at a final concentration of 500 μg/mL and subjected to probe sonication (Sonics Vibra™; Model No.VC505, New town, USA) at 30 W for 10 min (45 s pulses on and 15 s pulses off) cycles then cells were exposed to varying concentration (50–500 μg/mL) of CNPs NPs for 24 h. In each experiment, cells without CNPs were used as a control. Cellular morphology of CNPs treated, V-79 cells were analysed by phase contrast microscopy. V-79 cells are approved by OECD for conducting studies on genotoxicity, mutagenicity and repair of wide variety of DNA damaging studies. The cellular internalization of CNPs was also carried out via TEM. Live/dead assessment and membrane integrity analysis of CNPs treated V-79 cells were analysed by trypan blue exclusion assay with the help of Neubauer chamber and Propidium iodide (PI) uptake flow-cytometry based assay (FACS Canto™ II BD Biosciences, San Jose, CA, USA) (Strober, 2001; Tuschl and Schwab, 2004). The formation of intracellular reactive oxygen species (ROS) was determined by using fluorescence dye 2, 7-dichlorofluorescein diacetate (DCFH-DA) and excitation/emission wavelength of 485/525 nm in spectrophotometer (Molecular Devices Spectra Max5, USA) (Jain et al., 2017). Genotoxicity assessment of CNPs treated cells was determined by alkaline single cell gel electrophoresis assay popularly known as comet assay, employed for detection of DNA strand breaks (Cha et al., 2013).

Representative micrographs for surface topography were shown in Fig. 1. It is markedly demonstrated from SEM images (Fig. 1(a)) that CNPs are interconnected, small branched CNTs like appearance and spherical in shape. At lower magnification (inset of Fig. 1(a)), the synthesized CNPs are seemed agglomerated and assembled in sponge like assembly. The previously mentioned observations are also investigated by TEM analysis (Fig. 1 (b)). A typical fractal like, short branched (clearly seen in inset of Fig. 1(b)) and homomorphic CNPs are distinguished. The reason behind making chain may be due to the strong diffusive bonding and/or diffusive adhesion which is also responsible for mechanical stability (Rajeshwari and Dey, 2017). The average size of synthesized CNPs was found to be around 40 nm. Fundamentally, these images have been giving the impression of solid sphere with no free volume inside i.e. low porosity. This is also proved by PSD analysis discussed in next paragraph of this manuscript. The internalization of CNPs was measured with the help of TEM and photomicrographs illustrate the accumulation of CNPs in V-79 cells (Fig. 1(c–d)). In these images, V-79 cells were treated with 400 μg/mL of CNPs and no observable ultrastructural changes were found as compared to control after 24 h of exposure.

S_{BET} and PSD of synthesized CNPs was calculated by N_2 adsorption-desorption isotherms. Detail protocol for the analysis are described elsewhere (Singh and Verma, 2015a). The tabulated and isotherm data are labelled in Fig. 2(a). Isotherm is following type II pattern, which also endorses CNPs are finely divided and less porous ($V_{Total} = 0.0999$ cc/g) spherical solid structure which has been also confirmed by the TEM. Nonetheless, S_{BET} of CNPs was determined from the relative pressure range of 0.05–0.35 and found to be ~112 m^2/g and contain maximum mesoporosity (~58%). The diffraction pattern of synthesized CNPs was examined and obtained spectrum represents in Fig. 2(b). Examination procedure was same as described earlier (Singh and Verma, 2015b). The spectrum demonstrates two major diffraction peaks with at 25.5° for amorphous nature but also have little extent of parallel stacked graphene layers while, at 43° (low) confirms the crystalline honeycomb graphitic carbon structure of CNPs (Singh et al., 2018). The existence of surface functional groups in CNPs are exhibited in Fig. 2 (c). The spectra of CNPs reveal six prominent peaks which are found at nearly 640 cm^{-1}, 1050 cm^{-1}, 1600 cm^{-1}, 2340 cm^{-1}, 3000 $cm^{-1}$and 3600-3900 cm^{-1}.

These peaks are confirming the –C–H stretch of alkene/aromatic carbon, C–O stretch of alcoholic group, –C=O stretch of carboxylic acid, CO_2 from unburnt hydrocarbon present in soot, –C–H stretch of aliphatic carbon and –O–H stretch of adsorbed moisture phase respectively, present in synthesized CNPs (Singh et al., 2013, 2014, 2018). These functional groups may play an important role while binding other groups. A typical Raman