Nano-Structures & Nano-Objects 13 (2018) 30–35

Contents lists available at ScienceDirect

Nano-Structures & Nano-Objects

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

Novel nano-cocoon like structures of polyethylene glycol–multiwalled carbon nanotubes for biomedical applications

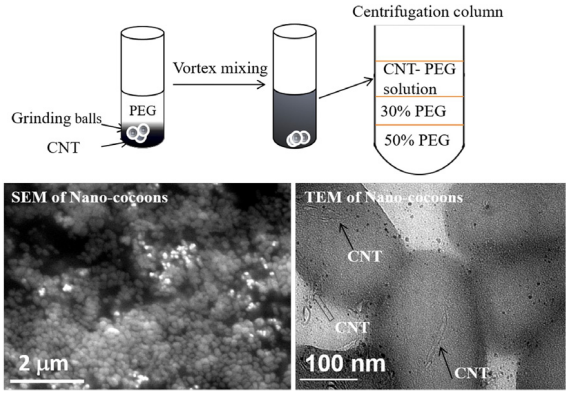
Hindumathi R. [a], Jagannatham M. [b], Prathap Haridoss [b,*], Chandra P. Sharma [c]

[a] Department of Biotechnology, Indian Institute of Technology Madras, Chennai - 600036, India
[b] Department of Metallurgical and Materials Engineering, Indian Institute of Technology Madras, Chennai - 600036, India
[c] Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Satelmond Palace, Poojappura, Trivandrum - 695012, India

HIGHLIGHTS

- PEG-CNT nanocomposite as structures similar to that of cocoons were synthesized.
- The synthesized nano-cocoons are hemo-compatible.
- Naturally anti-cancerous curcumin could be loaded onto the cocoons.
- The cocoon–curcumin complex could be uptaken by brain cancer cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 May 2017
Received in revised form 29 October 2017
Accepted 4 November 2017

Keywords:
PEG-MWCNT
Nano-cocoon
Nano biomaterial
Polymer nanocomposite

ABSTRACT

In this article, we report the synthesis and use of a novel drug delivery particle, based on bio-compatible polymer polyethylene glycol (PEG-400) and multi-walled carbon nanotubes (MWCNTs). MWCNTs in PEG-400 were broken into small tubes by vortex mixing with tungsten-carbide balls for about 15 h. Length separation of MWCNTs was then done using differential centrifugation with various concentrations of PEG-400. The separated MWCNTs in PEG solution were further pelletized using high speed centrifugation and re-dispersed in water. Novel cocoon like oval nanoparticles of about 100–200 nm size was observed in one of the centrifuged fractions. TEM shows that the cocoons primarily have PEG enclosing few MWCNTs. However, similar structures were not found when differential centrifugation was done without MWCNTs. These nano-cocoons are hemo-compatible, non-toxic to mice fibroblast cell lines (L929), have great potential to be used as nano biomaterials and they could be loaded with naturally anti-cancerous drug curcumin. The cocoon–curcumin complex is dispersible in saline and could be internalized by brain cancer cells (C6 glioma) while free curcumin dispersed in saline could not enter C6 glioma cells.

1. Introduction

Numerous studies are focused on drug-delivery properties of carbon nanotubes (CNTs), but these studies are limited by its toxicity [1–3]. The toxicity is attributed to several factors such as purity, size, dosage, surface properties and mode of exposure to biological

* Corresponding author.
E-mail address: prathap@iitm.ac.in (P. Haridoss).

https://doi.org/10.1016/j.nanoso.2017.11.001
2352-507X/

cells and tissues [4,5]. Functionalization with biocompatible materials can largely decrease the toxic effects of carbon nanotubes [6]. Still, the pharmacological and toxicology profile largely depends on the size of the CNTs and their state of aggregation inside the biological system [7,8]. Multi-walled carbon nanotubes (MWCNTs) with small length are less toxic than longer MWCNTs and single walled carbon nanotubes as they are less likely to get accumulated inside body parts causing chronic inflammation [9]. The focus of the present work has therefore been to produce and separate small sized MWCNTs and functionalize to make them more biocompatible. Studies focused on synthesis of MWCNTs of less than 1 μm are very few because purity level of small MWCNTs synthesized will be very less, since the formation of high percentage of other carbon nanoparticles along with it are very high. Alternatively, cutting of long MWCNTs followed by separation can be done. Sonication is known for efficient dispersion and cutting of length of MWCNTs, but prolonged sonication results in damage of the nanotubes walls and degradation of small nanotubes [10,11]. Vigorous mechanical beating can break the MWCNTs without affecting the nanostructure, and hence has been pursued in this work using prolonged vortex mixing aided by tungsten-carbide balls for production of large quantities of small MWCNTs. As addition of polyethylene glycol (PEG) could make CNTs bio-compatible [12], PEG has been used as both dispersion medium as well as separation medium. The process leads to the production of novel cocoon like structures which are PEG-MWCNT nanocomposites with biocompatibility properties.

As an essential component of regular food in Indian subcontinent, curcumin has many beneficial properties such as anti-inflammatory, anti-microbial, anti-oxidant and anti-carcinogenic properties where it is known to suppress proliferation of many tumor cells. But its bio-availability is limited, which can be improved by solubilizing in a bio-compatible hydrophilic polymer [13]. Hence, curcumin has been used in this work to study the possibility of adding a hydrophobic drug to these nanostructures.

2. Materials and methods

2.1. Materials used for preparation of nano-cocoons

Chemical Vapor Deposition made MWCNTs with purity level greater than 95% were procured from Chengdu Organic Chemicals Co. Ltd., China and used without any further purification. PEG-400 was procured from Merck. Tungsten-carbide balls purchased from Ant ceramics Ltd, India were used for the study.

2.2. Dispersion of carbon nanotubes and formation of nano-cocoons

1% of as received MWCNTs were dispersed in 15% PEG-400 for about 15 h using REMI CM-101 plus vortex mixer. Tungsten carbide balls are regularly used in grinding of CNTs using the process of ball milling, and is well established for shortening of nano structures including CNTs. This ability of grinding and shortening of CNTs is utilized in this study. The mechanical energy generated while tungsten carbide balls repeatedly hit the CNTs is utilized to shorten the nanotubes. Here we used tungsten carbide balls of 5 and 3 mm diameter in dispersing medium to obtain large number of small MWCNTs.

The mixture was then ultrasonicated for 10 min using Crest Ultrasonic Bath sonicator with combined 25 and 132 kHz frequencies. 5 ml of dispersed solution was added to the top of the differential centrifugation column with 30% and 50% PEG 400 solutions at the bottom. Centrifugation was done at 2000 rpm × 30 min and fractions of different concentration PEG 400 were separated, centrifuged at 2500 rpm × 20 min to remove the aggregates. The supernatant was diluted with distilled water at 1:2 ratio and centrifuged at 8000 rpm for 15 min for pellet or sediment formation. The sediment was diluted with distilled water, and pelletized again. This process was repeated twice for removing any trace PEG 400. Pellet obtained from 30% PEG 400 was collected separately and vacuum dried. The nano-structure of the particles in the pellet is similar to that of cocoons and henceforth termed as nano-cocoons. The above process of dispersion and separation was repeated without MWCNTs for comparison.

2.3. Curcumin addition

The cocoon pellet was re-dispersed in 1 ml distilled water and added with 1 ml of 4 mg/ml curcumin in ethanol. After 1 h, the mixture was pelletized using centrifugation, sediment was vacuum dried and weighed to quantify. Curcumin content was quantified by re-dispersing in ethanol and measuring the absorbance at 421 nm using uv–vis spectroscopy.

2.4. Electron microscopy and FTIR analysis

The cocoon pellets were re-dispersed in water using ultrasonication for 2 min and characterized using Quanta-400 Field emission scanning electron microscope (FE-SEM) operated at 30 kV and Transmission Electron Microscopy (TEM, Tecnai G^2) operated at 200 kV. Energy dispersive spectroscopy (EDS) and diffraction pattern were also obtained using Tecnai G^2 TEM. The Fourier Transformed Infra-Red (FTIR) spectra were obtained using Perkin Elmer Spectrum one: FT-IR Spectrometer of 1.0 cm^{-1} resolution from 500–4000 cm^{-1} range.

2.5. Particle size

1 mg/ml PEG-MWCNT solution (0.2% MWCNTs pre-dispersed in 15% PEG 400), 1 mg/ml cocoons, 0.5 mg/ml cocoon–curcumin (with 15% curcumin content) and 0.075 mg/ml curcumin (prepared from stock solution of 1 mg/ml curcumin in ethanol) in saline were probe sonicated for 10 min. The hydrodynamic particle size and polydispersity index (PDI) of cocoon and curcumin samples dispersed in saline was evaluated using Malvern zetasizer.

2.6. Hemolysis

Hemolysis was done as per procedure mentioned in ISO 10993-4:2002 (E). Blood from human volunteer was collected into the anticoagulant, ACD (Anticoagulant citrate dextrose) solution. 0.5 mg of each sample in 50 μl saline was exposed to 1 ml blood for 30 min in Environ shaker agitated at a speed of 70 ± 5 rpm, at 35 ± 2 °C. Three empty wells of polystyrene plates exposed with blood were taken as reference. The blood samples were centrifuged at 4000 rpm for 15 min and platelet poor plasma was aspirated. The total hemoglobin from the initial sample was measured using automatic haematology analyzer (Sysmex-K 4500). The free hemoglobin liberated in to the plasma after 30 min exposure was measured in each sample using Diode Array Spectrophotometer and the percentage hemolysis was calculated using the formula (Free Hb/Total Hb/1000) × 100.

2.7. Cytotoxicity-MTT assay on mouse fibroblast (L929) cells

Cocoon and cocoon–curcumin samples 25–100 μl each in triplets, were added to L929 cells seeded 1×10^5 cells per well for MTT assay. Polyethylenimine (PEI) 100 μg/ well was added as positive control and cell culture medium MEM (Minimum Essential Medium) as negative control. After 24 h, 100 μl of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) 0.5 mg/ml in phosphate buffered saline (PBS) was added to each well. The formazan crystals formed were dissolved in 200 μl DMSO (dimethyl sulfoxide) added to each well and quantified from the absorbance measured at 595 nm using Elisa plate reader.