BBA - General Subjects 1862 (2018) 2293–2303

Contents lists available at ScienceDirect

BBA - General Subjects

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

Proteomic investigation on bio-corona of functionalized multiwalled carbon nanotubes

Maria Nicoletti, Claudia Capodanno, Cristian Gambarotti, Elisa Fasoli*

Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, 20131 Milan, Italy

ARTICLE INFO

Keywords:
Carbon nanotubes
Proteomic fingerprinting
Mass spectrometry
Protein corona
Human plasma

ABSTRACT

Background: The formation of bio-corona, due to adsorption of biomolecules onto carbon nanotubes (CNTs) surface in a physiological environment, may lead to a modified biological "identity" of CNTs, contributing to determination of their biocompatibility and toxicity.

Methods: Multi-walled carbon nanotubes surfaces (*f*-MWCNTs) were modified attaching acid and basic chemical functions such as carboxyl (MWCNTs-COOH) and ammonium (MWCNTs-N) groups respectively. The investigation of interactions between *f*-MWCNTs and proteins present in biological fluids, like human plasma, was performed by electrophoretic separation (SDS-PAGE) and mass spectrometry analysis (nLC-MS/MS).

Results: A total of 52 validated proteins was identified after incubation of *f*-MWCNTs in human plasma. 86% of them was present in bio-coronas formed on the surface of all *f*-MWCNTs and 29% has specifically interacted with only one type of *f*-MWCNTs.

Conclusions: The evaluation of proteins primary structures, present in all bio-coronas, did not highlight any correlation between the chemical functionalization on MWCNTs and the content of acid, basic and hydrophobic amino acids. Despite this, many proteins of bio-corona, formed on all *f*-MWCNTs, were involved in the inhibitor activity of serine- or cysteine- endopeptidases, a molecular function completely unrevealed in the human plasma as control. Finally, the interaction with immune system's proteins and apolipoproteins has suggested a possible biocompatibility and a favored bio-distribution of tested *f*-MWCNTs.

General significance: Considering the great potential of CNTs in the nanomedicine, a specific chemical functionalization onto MWCNTs surface could control the protein corona formation and the biocompatibility of nanomaterials.

1. Introduction

Nanomedicine, born in the late 1990s, uses nanoparticles or nanostructured materials, characterized by nanoscale dimensions (from 1 to 1000 nm), for drug and gene delivery. These nanomaterials are able to interact with physiological biomolecules in the blood, in organs, in tissues or in cells, improving the crossing of biological tissue, the accumulation into tumor cells and the drugs bioavailability [1, 2].

The aims of Nanomedicine are numerous such as the monitoring and the control of human biological activities, the control and the repair of pathological conditions. The main research efforts combine molecular medicine and biomedical engineering to obtain nanomaterials useful for novel therapeutic devices and for Imaging tools [3]. In the field of Nanopharmaceutical, the first generation of drugs delivery systems was nano-sized drug carriers like liposomes, formed by physiological biomolecules [4, 5]. The second generation was polymeric nanostructures, directly conjugated with drugs and proteins, multi component polyplexes and polymeric dendrimers [6–14].

Recently, carbon nanotubes (CNTs) have been studied in nanomedicine as drug delivery systems: they are tubular carbon allotropes, formed by layers of graphene arranged in two-dimensional hexagonal lattice [15]. The number of graphene sheets, folded into cylindrical structure, defines Single-Walled CNTs (SWCNTs) and Multi-Walled CNTs (MWCNTs) [16]. CNTs appear to be promising materials for many biomedical applications like gene and drug delivery systems specially in cancer therapy, nanoscaffold for tissue regeneration, biosensors for diagnostics and biomedical imaging [17]. In the first case, CNTs hydrophobic structure favors their surface functionalization and the conjugation with therapeutic molecules (drugs, proteins, antibodies, DNA, enzymes, etc.), acting as an excellent vehicle for drug able to improve the cellular internalization without any induced inactivation [18]. Kumar et al. have reported an overview on both SWCNTs and

* Corresponding author.
E-mail address: elisa.fasoli@polimi.it (E. Fasoli).

https://doi.org/10.1016/j.bbagen.2018.07.024
Received 4 May 2018; Received in revised form 18 July 2018; Accepted 20 July 2018
Available online 24 July 2018

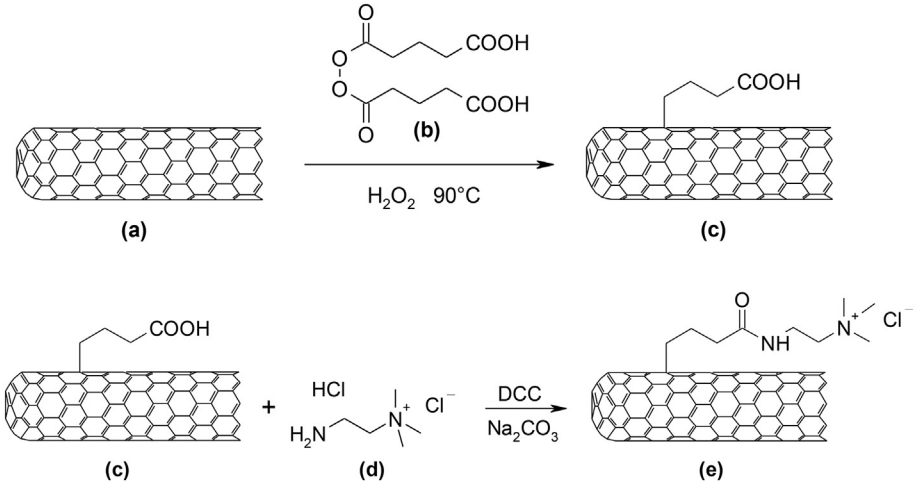

Fig. 1. Side-wall functionalization of MWCNTs by glutaryl peroxide (from a to c) and side-wall functionalization of MWCNTs by ammonium salt (from c to e).

MWCNTs application as drug delivery systems in cancer therapy and in anti-inflammatory treatment [19–27].

Despite their promising potentiality, CNTs biocompatibility and bioavailability are recently investigated, focusing efforts on specific factors able to influence them like morphology, physical features (size, shape), chemical properties (charge and/or agglomeration state), purity and presence of metal impurities [28, 29]. For this reason, CNTs effects on human health are nowadays evaluating: for example the effect of CNTs deposition in the respiratory system is deeply studied for their probable correlation with inflammatory processes and carcinogenesis [30–34]. Moreover, CNTs are able to cross the hematoencephalic barrier, favoring oxidative stress and lesions in neuronal cells, and for this reason they are correlated with development of neurodegenerative diseases [35, 36]. Finally recent in vivo studies on mice have revealed that the low degree of SWCNTs purity could increase the release of free radicals during oxidative stress [37].

The chemical functionalization on CNTs surface could play an important role both to improve their biocompatibility and to reduce their toxicity [38–41]. In fact, CNTs functionalization may influence the interaction with physiological proteins after administration, modulating nanostructures' biocompatibility and any cytotoxic effects. The binding with proteins, present in biological fluids and tissues, contributes to form the protein corona or bio-corona on CNTs surface, required to prevent immune responses [42–44].

Considering the CNTs biocompatibility, our research aimed to investigate the interactions between functionalized MWCNTs (*f*-MWCNTs) and proteins present in biological fluids, like human plasma (HP), using a proteomic approach based on electrophoretic separation (SDS-PAGE) and on mass spectrometry (nLC-MS/MS) for proteins identification. For this purpose, two preliminary steps were performed to optimize the final experimental procedure with HP: firstly the standardization of incubation protocol between *f*-MWCNTs and standard proteins solution, secondly the evaluation of interaction between *f*-MWCNTs and a complex protein solution extracted from *Escherichia coli*. The initial steps aimed to develop the most performant protocol, optimizing the modality of incubation (e.g. temperature, time) and of proteins concentration, and to investigate the nanotubes' interaction with a complex proteome. The lack of such experimental data in literature was mostly due to the characterization of protein corona normally after in vivo administration of functionalized carbon nanotubes [45].

2. Material and methods

2.1. Multi-walled Carbon Nanotubes (MWCNTs) preparation

The Multi-Walled Carbon Nanotubes (MWCNTs) were prepared by the typical Chemical Vapour Deposition (CVD) protocol using ethylene, as carbon source [46], and they were characterized by TEM microscopy, Thermo Gravimetric Analysis (TGA), Inductively Coupled Plasma (ICP) analysis and X-ray photoelectron spectroscopy (XPS).

TEM observations were performed with a Philips CM200 field emission transmission electron microscope; the sample of nanotubes, dispersed in 4% aqueous SDS, were deposited an a holey carbon films on 200 mesh copper grids and then observed under accelerated voltage of 200 kV. ICP analyses were performed on a Perkin Elmer Optima 8300. TGA curves were recorded using a Perkin Elmer STA 6000, the analyses were performed in air from 35 °C to 900 °C with a continuous heating rate of 10 °C/min.

XPS spectra were obtained by using an M-probe apparatus (Surface Science Instruments). The source was monochromatic Al Kα radiation (1486.6 eV). A spot size of 200 × 750 μm and a pass energy of 5 eV were used. Fits were performed using pure Gaussian peaks, Shirley's baseline, and without any constraints.

In order to introduce the carboxylic acid functions, the nanotubes were functionalized to the corresponding MWCNTs-COOH (c) by in situ thermal decomposition of glutaryl peroxide (b) [47], which was previously prepared from glutaric anhydride, following the procedure reported in literature (Fig. 1) [48].

Particularly, 1 g of pristine MWCNTs (a) was dispersed in 3.8 mmol glutaryl peroxide solution (b) in 250 ml of H_2O. The resulting mixture was sonicated in an ultrasound bath (240 W, 2.5 l) for 10 min, heated and stirred for 7 h at 90 °C. The suspension was filtered on a sintered glass filter and MWCNTs-COOH (c) were carefully washed with hot water, filtered and dried at 90 °C overnight.

The nanotubes ammonium salt MWCNTs-N (e) were prepared by amidation of the functionalized MWCNTs-COOH (c) with the modified amine (d), in the presence of *N,N'*dicyclohexylcarbodiimide (DCC) and sodium carbonate as reported in Fig. 1.

The pristine MWCNTs, MWCNTs-COOH and MWCNTs-N were further ultrasonicated with an ultrasonic cell disruptor Branson Sonifier 250 equipped with a Tapped Bio Horn 101–147-037 with 1/2 in. of diameter. The ultrasound treatment was conducted by dispersing the nanotubes in water (1% w/w), with the ultrasound power set on 20 W