Biomaterials 32 (2011) 1121–1129

Contents lists available at ScienceDirect

Biomaterials

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

In vitro comparison of the photothermal anticancer activity of graphene nanoparticles and carbon nanotubes

Zoran M. Markovic [a,1,2], Ljubica M. Harhaji-Trajkovic [b,2], Biljana M. Todorovic-Markovic [a], Dejan P. Kepić [a], Katarina M. Arsikin [c], Svetlana P. Jovanović [a], Aleksandar C. Pantovic [c], Miroslav D. Dramićanin [a], Vladimir S. Trajkovic [c,*]

[a] Vinca Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia
[b] Institute for Biological Research, University of Belgrade, Belgrade, Serbia
[c] Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Dr. Subotica 1, Belgrade 11000, Serbia

ARTICLE INFO

Article history:
Received 24 September 2010
Accepted 15 October 2010
Available online 10 November 2010

Keywords:
Graphene
Carbon nanotubes
Cancer
Hyperthermia
Apoptosis

ABSTRACT

The present study compared the photothermal anticancer activity of near-infrared (NIR)-excited graphene nanoparticles and carbon nanotubes (CNT). Despite lower NIR-absorbing capacity, suspension of polyvinylpyrrolidone-coated graphene sheets exposed to NIR radiation (808 nm, 2 W/cm^2) generated more heat than DNA or sodium dodecylbenzenesulfonate-solubilized single-wall CNT under the same conditions. Accordingly, graphene nanoparticles performed significantly better than CNT in inducing photothermal death of U251 human glioma cells in vitro. The superior photothermal sensitivity of graphene sheets could be largely explained by their better dispersivity, which has been supported by a simple calculation taking into account thermodynamic, optical and geometrical properties of the two type of carbon nanoparticles. The mechanisms of graphene-mediated photothermal killing of cancer cells apparently involved oxidative stress and mitochondrial membrane depolarization resulting in mixed apoptotic and necrotic cell death characterized by caspase activation/DNA fragmentation and cell membrane damage, respectively.

1. Introduction

The selective thermal ablation of malignant tissue at temperatures above 40 °C is a feasible alternative treatment option when surgical resection is not possible [1,2]. Unfortunately, major thermal ablative strategies such as laser, ultrasound, microwave or radio frequency treatment do not possess inherent specificity for malignant cells. Therefore, the selective sensitization of malignant cells to irradiation is required to increase efficiency and reduce toxicity of thermal ablation. One of the promising strategies is photothermal therapy, in which nanoparticles such as carbon nanotubes (CNT) or gold nanoparticles are used to convert near-infrared (NIR) radiation to vibrational energy, thus generating heat sufficient for cancer cell killing [3,4]. The use of NIR light in the 700-1100-nm range for the induction of hyperthermia is particularly attractive, because biological systems mostly lack chromophores that absorb in the NIR region [5,6]. Hence, lesions can be treated without the need for direct access to the tumor site, with the NIR light effectively and safely penetrating normal tissue and selectively destroying cancer cells to which the nanoparticles are attached.

CNT are cylindrical structures up to hundreds of nanometers in diameter and up to few micrometers in length, consisting of atomically thin sheets of carbon known as “graphene”. Graphene was merely considered as part of graphite’s crystal structure until 2004, when graphene layers isolated by mechanically peeling sheets off graphite crystals have been shown to possess some surprising electrical properties [7]. During the past few years, graphene’s unique electronic structure and properties have started to threaten the dominance of CNT in potential applications from electronics to sensors. Moreover, the in vitro toxicity of graphene towards human cells appears to be lower than that of CNT [8], thus supporting its possible use in biomedicine. Accordingly, graphene oxide nanoparticles have been considered as potential cancer biomarker sensors and nanoplatforms for cancer-selective drug delivery [9,10]. On the other hand, while photothermal anticancer effects of CNT have been well-documented [11–17], the possibility of direct tumor cell killing by graphene-based nanoparticles has remained largely unexplored. Namely, only one very recent study has so far demonstrated the ability of intravenously injected polyethylene glycol-coated pure graphene sheets to reduce tumor size in mice by

* Corresponding author. Tel.: +381 11 3643 233; fax: +381 11 3643 235.
E-mail addresses: zormark@vinca.rs (Z.M. Markovic), vtrajkovic@eunet.rs (V.S. Trajkovic).
[1] Tel.: +381 11 2455 451; fax: +381 11 3440 100.
[2] These authors equally contributed to the work.

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doi:10.1016/j.biomaterials.2010.10.030

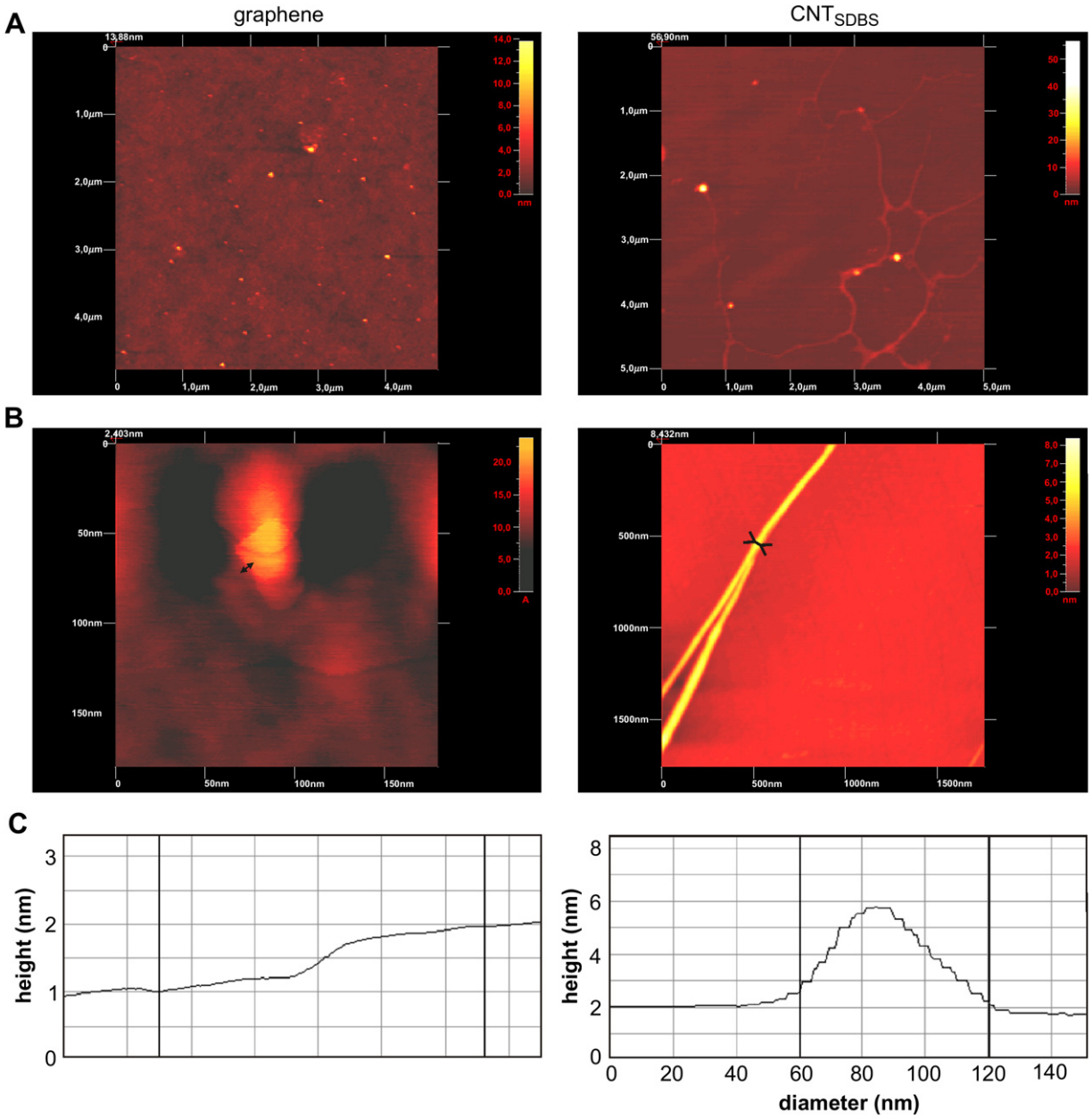

Fig. 1. AFM analysis of carbon nanomaterials. (A) Large-scale and (B) small-scale AFM images of graphene nanoparticles and CNT_{SDBS} are presented together with (C) the surface profile of the individual nanoparticles.

NIR-stimulated hyperthermia [18]. However, it has not been reported how the photothermal efficiency of graphene compares to that of carbon nanotubes, or which cell death mechanisms are responsible for graphene-mediated photothermal killing of cancer cells.

In the present study, we used an in vitro system for NIR-induced hyperthermia to directly compare the photothermal anticancer efficiency of graphene nanoparticles and carbon nanotubes, as well as to investigate the type and molecular mechanisms of graphene-mediated photothermal cancer cell death.

2. Materials and methods

2.1. Preparation and characterization of graphene nanoparticles and carbon nanotubes

A stable water suspension of graphene nanoparticles was prepared by aqueous-phase exfoliation of graphite in the presence of non-ionic/non-toxic macromolecule polyvinylpyrrolidone (PVP) [19], while single-wall CNT were made water-soluble by functionalization with DNA or anionic detergent sodium dodecylbenzenesulfonate (SDBS), as previously described [20,21]. Briefly, 500 mg of crystalline graphite powder (synthetic, 6 μm; Timcal, Bodio, Switzerland) were suspended in 100 ml of deionized water containing 1 g PVP (Sigma–Aldrich, St. Louis, MO), while 50 mg of single-wall CNT (95%; BuckyUSA, Houston, TX) were added to 100 ml of deionized water containing 1 mg of heat-denatured single-stranded DNA or 2 mg SDBS (both from Sigma–Aldrich, St. Louis, MO). The graphene and CNT suspensions were sonicated in a 750 W ultrasonic bath for 3 h, centrifuged at 4000 rpm for 1 h to remove large aggregates, and the resulting clear colloids were collected. The total carbon particle concentration in the obtained suspensions was determined using gravimetric method, by drying 10 ml of colloid at 60 °C in air and measuring the dry residue. The following concentrations were obtained: 22 μg/ml (graphene/PVP-G_{PVP}), 25 μg/ml (CNT_{DNA}) and 180 μg/ml (CNT_{SDBS}). Atomic force microscopy (AFM) measurements were performed using an AFM microscope (Quesant Instrument Corp., Agoura Hills, CA) operating in tapping mode in air at room temperature. Suspensions of carbon nanoparticles were deposited on silicon substrate by spin coater and the images were obtained after drying in air and vacuum annealing at 60 °C, using standard silicon tips (NanoAndMore GMBH, Wetzlar, Germany) with the force constant of 40 N/m. The UV–vis spectra of the nanocarbon-based suspensions were scanned within the wavelength range of 500–1100 nm using Avantes UV–vis