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Structure-dependent photothermal anticancer effects of carbon-based photoresponsive nanomaterials

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ABSTRACT

Here, we report the effect of structure on the biological properties of photoresponsive carbon nanomaterials. Poloxamer 407-functionalized single-walled carbon nanotubes (PSWCNT) and poloxamer 407-functionalized graphene nanosheets (PGNS) exhibited similar physical stability and heating capacities after irradiation with an 808 nm near-infrared (NIR) laser. Despite sharing common physical properties, the cellular uptake of the PSWCNT and PGNS differed significantly. Cancer cells treated with PGNS took up a higher quantity of the nanosheets than of the PSWCNT and displayed a higher rate of cancer cell killing upon laser irradiation. Structure of carbon nanomaterials also affected the in vivo behaviors. PGNS could circulate in the blood 2.2 times longer than that of the PSWCNT. PGNS accumulated in the SCC tumor tissues to a greater degree than did PSWCNT over 7 days. NIR irradiation resulted in the complete ablation of tumor tissues in the PGNS-treated group but not in the other groups. After NIR irradiation, 100% of the PGNS-treated and NIR-irradiated mice survived until day 70. These results suggest the importance of structure in controlling the in vivo behaviors of carbon nanomaterials. Moreover, the results indicate the structural advantages of nanosheets over nanotubes in the enhancement of photothermal anticancer effects.

1. Introduction

Although remarkable progress has been made in cancer treatment, conventional strategies, such as surgical resection, chemotherapy, radiotherapy, and their combinations have shown limited success toward cancer eradication over the past few decades. Photothermal therapy (PTT) for the treatment of solid tumors recently emerged as an attractive alternative approach to converting absorbed light into local heating through nonradiative mechanisms [1]. Anticancer PTT is advantageous over surgical methods and chemotherapy because it permits spatial and temporal control, is minimally invasive, and results in few complications [2–4].

Near-infrared (NIR) light (700–1100 nm) is used in PTT because it penetrates deeply into the tissue and is absorbed only to a small degree by normal tissue [5,6]. Light-absorbing agents that display a high degree of absorption in the NIR are generally involved in PTT processes to facilitate energy conversion from light to heat in localized tumor tissues. Experimentally tested photoresponsive light absorbers include gold [2,7], and carbon nanomaterials such as carbon nanotubes [8] and graphene [9,10].

Single-walled carbon nanotubes (SWCNT) and graphene nanosheets have been studied for their utility in photothermal cancer treatment. Intratumoral injection of phospholipid-polyethylene glycol-coated SWCNT [8] or polyethylene glycol-conjugated SWCNT [11] has been reported to destroy tumors upon irradiation with NIR laser. Intravenous injection of polyethylene glycol-conjugated graphene nanosheets [9] has been shown to provide NIR laser-induced antitumor photothermal effects. Polyvinylpyrrolidone-coated graphene nanosheets were reported to induce photothermal death of human glioma cells [12].

Although previous studies described the potential utility of SWCNTs and graphene-based nanosheets in PTT, it is little studied whether the different geometry of these carbon-based nanomaterials could influence the biological and pharmacological effects. In this study, using amphiphilic triblock copolymer-functionalized SWCNT and graphene nanosheets, we tested whether the structure of carbon-based nanomaterials could affect their physical properties, in vivo fates, and photothermal anticancer effects.

2. Materials and methods

2.1. Preparation of carbon nanomaterials functionalized with poloxamer 407

Graphene nanosheets functionalized with poloxamer 407 (PGNS) were prepared by the aqueous-phase exfoliation of graphite in the presence of poloxamer 407 (Sigma–Aldrich, St. Louis, MO, USA), as described previously [13]. In brief, 50 mg

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graphite powder (Sigma–Aldrich) was suspended in 50 mL of a 1% w/v poloxamer 407 solution. The dispersions were sonicated over ice for 2 h using a horn ultrasonicator equipped with a 13 mm diameter probe (VCX 500, Sonics & materials, Inc., Newtown, CT, USA). The solutions were then centrifuged at 16,000× *g* for 30 min twice to remove aggregates. The resulting PGNS supernatants were collected and stored at 4 °C until use.

SWCNT noncovalently coated with poloxamer 407 (PSWCNT) were prepared by incubating SWCNT in the presence of poloxamer 407 (Sigma–Aldrich), as described previously [13]. Briefly, SWCNT (Nanocs Inc., New York, NY, USA) were suspended in 50 mL of a 1% w/v poloxamer 407 solution. These dispersions were sonicated over ice for 2 h using a horn ultrasonicator equipped with a 13 mm diameter probe (VCX 500). The dispersions were then centrifuged at 16,000× *g* for 30 min twice to remove aggregates. The resulting PSWCNT supernatants were then collected and stored at 4 °C until use.

2.2. Characterization of the PGNS and PSWCNT

The UV–vis spectra of the PGNS and SWCNT were recorded over the range 200–810 nm using a UV–vis spectrophotometer (UV-3100, Shimadzu Corp, Tokyo, Japan). The concentrations of carbon in the PGNS and PSWCNT suspensions were determined from the extinction coefficient at 660 nm (6600 L g^{-1} m^{-1}) [14], and at 808 nm (4650 L g^{-1} m^{-1}) [15], respectively. The sizes and morphologies of the PGNS and PSWCNT were examined by transmission electron microscopy (TEM, JEM1010, Jeol Ltd, Tokyo, Japan).

2.3. Laser irradiation and photothermal imaging

Carbon nanomaterial-based suspensions of PGNS or PSWCNT were serially diluted with distilled water and irradiated using an 808 nm continuous wave NIR diode laser beam (BWT Beijing LTD, Beijing, China) with an output power of 1.2 W. The temperature and photothermal images of the carbon nanomaterial-based suspensions during laser irradiation were recorded using an infrared thermal imaging system every 30 s (FLIR T420, FLIR Systems Inc., Danderyd, Sweden).

2.4. In vitro cellular uptake study

The cellular uptakes of PGNS or PSWCNT were determined using confocal microscopy. The cellar uptake was visualized by labeling the PGNS and PSWCNT with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-poly(ethyleneglycol) 5000-Cy5.5 (DSPE-PEG_{5000}-Cy5.5) lipids. The synthesis of DSPE-PEG_{5000}-Cy5.5 and the labeling of PGNS or PSWCNT with the lipid are described in the Supplementary Information. Murine SCC7 squamous carcinoma cells (American Type Culture Collection, Rockville, MD, USA) were cultured in Dulbecco's modified Eagle medium (Gibco BRL life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum and 100 units/mL penicillin plus 100 μg/mL streptomycin (complete DMEM media). SCC7 cells were seeded onto a cover glass at a density of 1×10^5 cell/well in 12-well plates. When the cells reached 70% confluence, DSPE-PEG_{5000}-Cy5.5-labeled PGNS or PSWCNT suspensions with a carbon concentration of 10 μg/mL were added to each well. After 24 h, the cells were washed by cold phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 15 min, and stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI, Sigma–Aldrich). The fluorescence of the cells was observed using a confocal laser scanning microscope (LSM 5 Exciter; Carl Zeiss, Inc., Jena, Germany). Flow cytometry measurements were conducted by harvesting the cells and washing thrice with cold PBS containing 2% fetal bovine serum. The cells were analyzed using a BD FACSCalibur flow cytometer using the Cell Quest Pro software (BD Bioscience, San Jose, CA, USA).

2.5. Quantitative cell viability assay following NIR laser irradiation

SCC7 cells were seeded onto 12-well plates at a density of 1×10^5 cell/well. The following day, cells were treated with PGNS or PSWCNT at a carbon concentration of 20 μg/mL. After 24 h incubation at 37 °C, the cells were washed twice with cold PBS and resuspended in complete DMEM media. The cell suspensions were irradiated with an 808 nm continuous-wave NIR diode laser at an output power of 1.2 W for various exposure periods. In some experiments, untreated SCC7 cell suspensions were mixed with PGNS or PSWCNT at various carbon concentrations and irradiated with the NIR laser. Immediately after irradiation, the cells were diluted 10-fold using complete DMEM media and transferred to 96-well plates for the cell viability assays. The cell viability was quantified using a Cell Counting Kit-8™ (CCK8) according to the protocol provided by the manufacturer (Dojindo Molecular Technologies, Inc., Rockville, MD, USA). The values are expressed as a percentage of the cell viability measured in the control groups.

2.6. Animals

In vivo experiments were conducted using five week old female Balb/c athymic nude mice supplied by Orient Bio. Lab. Animal Inc. (Seungnam, Kyonggi-do, South Korea, approved animal experimental protocol number SNU-130129-3-1). Animals were raised under standard pathogen-free conditions at the animal center for pharmaceutical research in the Seoul National University. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Seoul National University.

2.7. Pharmacokinetic study

The pharmacokinetic profiles of the PGNS and PSWCNT were determined by intravenously administering to the mice DSPE-PEG_{5000}-Cy5.5-labeled PGNS or PSWCNT at a carbon dose of 5 mg/kg. The blood samples were collected at various time points after dosing. The fluorescence intensities in the blood were measured using an eXplore Optix System (Advanced Research Technologies Inc., Montreal, Canada). The excitation and emission spots were raster-scanned in 1 mm steps over the region of interest to generate the emission wavelength scans. A 670 nm pulsed laser diode was used to excite Cy5.5 molecules. Long wavelength fluorescence emission (600–700 nm) was detected using a fast photomultiplier tube (Hamamatsu Photonics, Hamamatsu, Japan) and a time-correlated single photon counting system (Becker and Hickl GmbH, Berlin, Germany). Finally, the non-compartmental pharmacokinetic parameters were calculated using the software program WinNonlin™ (Scientific Consulting Inc., Lexington, KY, USA). The mean residence time (MRT) was calculated using the non-compartmental method by dividing the area under the momentum curve with area under the curve (AUC).

2.8. In vivo molecular imaging

The biodistributions of PGNS or PSWCNT in tumor-bearing mice were examined by molecular imaging. Mice were subcutaneously inoculated at the right dorsal side with 1×10^6 SCC7 cells, and tumors were allowed to grow over time. Suspensions of the carbon-based nanomaterials, DSPE-PEG_{5000}-Cy5.5-labeled PGNS and PSWCNT, were intravenously administered at a carbon dose of 5 mg/kg to the SCC7-bearing mice. At various time points post-dose, the tumor tissue distribution of the fluorescent PGNS or PSWCNT was assessed using the eXplore Optix System, as described above.

2.9. In vivo photothermal tumor ablation study

The photothermal anticancer effects of PGNS and PSWCNT were tested using SCC7 tumor-bearing nude mice. Five week old athymic nude mice (Orient Bio, Inc.) were subcutaneously injected at the dorsal right side with 1×10^6 SCC7 cells. When the tumor volume reached 50–80 mm^3, the mice were subjected to intravenous administration of PGNS or PSWCNT at the same carbon dose of 5 mg/kg. One day post-administration, the mice were anesthetized and positioned in a mouse holder. The tumor sites were irradiated for 3 min with an 808 nm continuous wave NIR laser at an output power of 1.2 W. Light-induced temperature changes in the tumor region were recorded using a real-time infrared thermal imaging system (FLIR T420, FLIR Systems Inc., Danderyd, Sweden). The tumor sizes were measured in two dimensions using a slide caliper, and the tumor volume was calculated according to the equation $a \times b^2 \times 0.5$, where a is the largest and b is the smallest diameter. In some experiments, the tumor tissues were extracted after irradiation and fixed in 4% paraformaldehyde in PBS, embedded in paraffin, and sectioned at a thickness of 6 μm. The slides of the tumor tissue were stained with hematoxylin and eosin and were observed using optical microscopy. Survival was evaluated by monitoring the mice daily in each group after the administration of PGNS or PSWCNT.

2.10. Statistics

ANOVA techniques were used to statistically evaluate the experimental data. The Student–Newman–Keuls test was used as a post-hoc test. All statistical analyses were performed using the SigmaStat software (version 3.5, Systat Software, Richmond, CA, USA), and a p-value of <0.05 was considered significant.

3. Results

3.1. Characterization and thermal conductivity of carbon-based nanomaterials

PSWCNT and PGNS provided similar dispersions, UV spectra, and photothermal conductivity values (Fig. 1A). Both PSWCNT and PGNS were freely dispersed in fetal bovine serum (Fig. 1B). The absorption patterns of the UV–vis spectra of the two carbon-based nanomaterials showed peaks at 260–270 nm and tapered down at longer wavelengths (Fig. 1C). TEM measurements revealed the unique nanotube and nanosheet structures for PSWCNT (Fig. 1D) and PGNS (Fig. 1E), respectively. The photothermal properties of PSWCNT were similar to those of PGNS (Fig. 2). Regardless of the carbon-based nanomaterial structure, a dose-dependent increase in the temperature was observed in suspensions of PSWCNT or PGNS in proportion to the NIR laser (808 nm) irradiation time. Following 5 min NIR irradiation, the temperatures of the nanomaterial suspensions prepared with a carbon concentration of