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Palladium nanoparticle-decorated 2-D graphene oxide for effective photodynamic and photothermal therapy of prostate solid tumors

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A B S T R A C T

Intratumoral injection of nanoparticles is a viable alternative for treating solid tumors. In this study, we used intratumorally-injected palladium nanoparticle (Pd NP)-decorated graphene oxide (GO) (GO-Pd NPs) for the treatment of solid prostate tumors. GO was synthesized using the modified Hummer's method and GO-Pd NPs were prepared using the one pot synthesis method. Studies on physicochemical characterization and *in vitro/in vivo* anticancer properties were performed using GO-Pd NPs. Successful preparation of GO-Pd NPs was confirmed by transmission electron microscopy, Fourier transform infrared spectroscopy, energy dispersive X-ray spectroscopy, and X-ray photoelectron spectroscopy. Compared to GO or Pd NPs alone, GO-Pd NPs showed higher cytotoxic effects in prostate cancer 3 (PC3) cells. Irradiation of treated cells with near infrared (NIR) laser considerably enhanced apoptosis induced by synergistic photothermal effect and reactive oxygen species (ROS) generation. Intratumorally-injected GO-Pd NPs showed promising *in vivo* localized distribution, photothermal ablation, and anti-tumor effects in the PC3 xenograft mouse model. Furthermore, the minimal organ toxicity of GO-Pd NPs was an added advantage. Hence, GO-Pd NPs could be a potential formulation for localized treatment of prostate solid tumors.

1. Introduction

Currently, nanoparticle-based therapy is being rigorously investigated for its anti-cancer potential. The ability to harbor chemotherapeutic agents and the possibility of performing surface modifications for stable and targeted drug delivery have been extensively exploited in nanoparticle-mediated cancer therapy [1,2]. In solid tumors, the hyperpermeability of blood vessels allows accumulation of nanoparticle-based cancer therapeutics at tumor sites via a phenomenon called "enhanced permeability and retention (EPR)" [3,4]. However, the inability of the nanoparticles to penetrate into the hypoxic tumor core leads to inefficient therapy and development of drug resistance [5,6]. Therefore, intratumoral injection might favor distribution of these nanosystems in solid tumors for effective anti-tumor therapy.

Chemotherapy inhibits or alters different molecular pathways related to angiogenesis, proliferation, and metastasis. Alternatively, phototherapy (photodynamic and photothermal) causes cancer cell apoptosis via reactive oxygen species (ROS)-induced mitochondrial dysfunction and thermal ablation of cells [7]. A combination of photodynamic therapy (PDT) and photothermal therapy (PTT) can synergistically improve cancer therapy [8,9]. Recently, various materials have been developed for PDT and PTT of cancers, including carbon-based nanomaterials (carbon nanotubes [10] and nano-graphene [11,12]), metal nanostructures (gold nanosystems [13–15] and palladium (Pd) nanosheet [16]), and copper sulfide [17,18] or selenium nanoparticles [19]. Nano graphene oxide (GO) is a two-dimensional carbon allotrope that induces ROS generation and near infrared (NIR) light-induced photothermal effect, following uptake by cancer cells [20,21]. In contrast, Pd nanoparticles (Pd NPs) are widely applied as catalytic materials for hydrogen storage and sensing, but have limited applications in biomedicine [22]. Few reports suggest that Pd NPs possess potential anticancer effects based on PDT and PTT [23,24]. Therefore, a combination of Pd NPs and GO might provide synergistic anticancer effects. Until now, reports on the use of Pd NP-decorated GO (GO-Pd NPs) for anticancer therapy were lacking. Here, for the first

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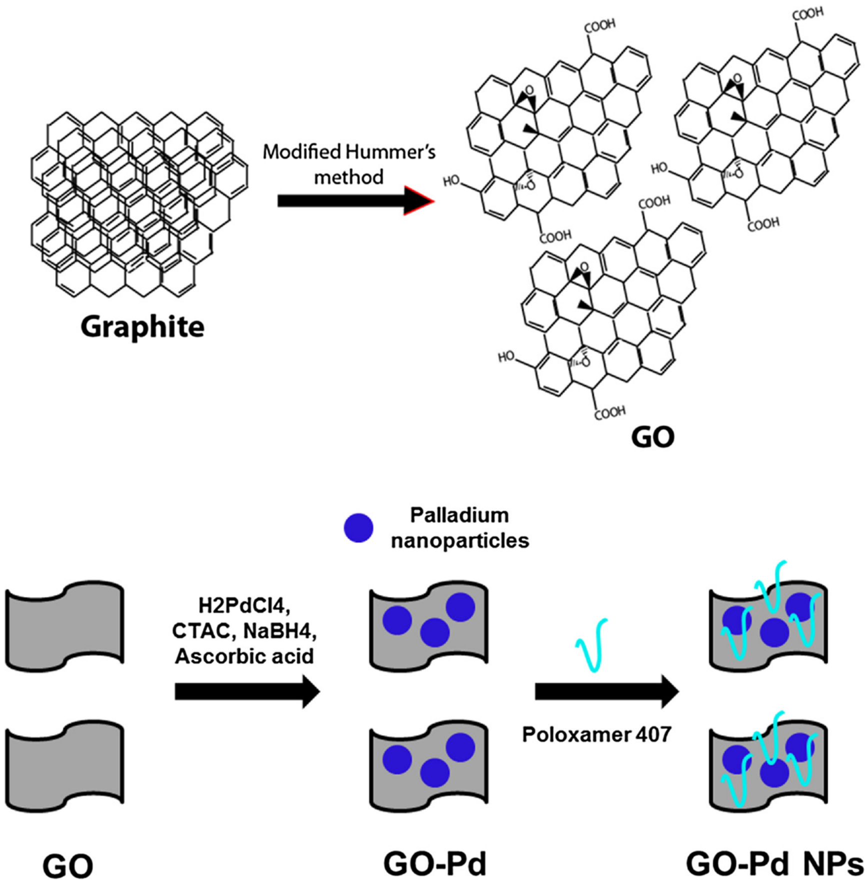

Fig. 1. Schematic representation of the synthesis of GO-Pd NPs.

time, we used GO-Pd NPs for the treatment of solid prostate tumors and evaluated their PDT and PTT-based anti-tumor effects.

2. Materials and methods

2.1. Materials

Graphite flakes were obtained from Alfa Aesar, Thermo Fisher Scientific (Waltham, MA, USA). Fluorescein-5(6)-isothiocyanate (FITC), palladium chloride ($PdCl_2$), cetyltrimethylammonium chloride (CTAC), sodium borohydrate ($NaBH_4$), and ascorbic acid were purchased from Sigma-Aldrich (St Louis, MO, USA). LysoTracker Red was procured from Invitrogen/Molecular Probes, Inc. (Eugene, OR, USA).

2.2. Synthesis of GO-Pd NPs

GO was synthesized using the modified Hummer's method [25]. Pd NPs were synthesized using the procedure reported by Wang et al. [26] A slight modification of this method was used for one-pot synthesis of GO-Pd NPs. Briefly, aqueous H_2PdCl_4 solution (0.01 M) was prepared by dissolving $PdCl_2$ (0.25 mmol) in HCl (0.02 M, 25 mL) solution at 50 °C. For the synthesis, 0.25 mL H_2PdCl_4 (0.01 M) and 9.75 mL CTAC (0.1 M) were mixed, followed by the addition of 0.60 mL ice-cold $NaBH_4$ (0.01 M) solution under vigorous stirring. This solution was then incubated undisturbed for 2 h at room temperature and used later as the seed solution. Pd NPs were grown onto GO by adding the seed solution to the mixture of GO (300 µg), 9.70 mL CTAC (4.5 mM), and 0.30 mL H_2PdCl_4, followed by addition of 0.10 mL ascorbic acid (0.1 M). The resulting dispersion was gently inverted for 10 s and maintained undisturbed for 6 h at room temperature. Finally, the prepared GO-Pd NPs were retrieved by 10 min centrifugation at 10,000 rpm followed by subsequent distilled water washes for removal of unreacted or excess chemicals. Pd NPs were synthesized using the same procedure except for the addition of GO.

2.3. Characterization of GO, Pd NPs, and GO-Pd NPs

Particle size, polydispersity index (PDI), and zeta potentials of GO, Pd NPs, and GO-Pd NPs were determined using a Nano-S90 ZetaSizer (Malvern Instruments, Malvern, UK). Morphology was characterized using a sample loaded carbon-coated copper grid in transmission electron microscopy (TEM, H7600, Hitachi, Japan). Ultraviolet (UV)/visible spectra of GO, Pd NPs, and GO-Pd NPs were documented using a UV/vis spectrophotometer (PerkinElmer U-2800, Waltham, MA, USA). Fourier transform infrared (FTIR) spectroscopy analyses were performed using a Thermo Scientific Nicolet Nexus 670 FTIR spectrophotometer (Thermo Scientific, Waltham, MA, USA). Solid state characterizations were performed using an X-ray diffractometer (X'Pert PRO MPD diffractometer, Almelo, Netherlands). The energy-dispersive X-ray spectra (EDS) and scanning electron microscopy images (SEM) were surveyed using a field emission scanning electron microscope (FESEM, S4800, Hitachi Co., Japan) equipped with an EDS (Jeol JXAe840). Surface element compositions were determined by X-ray photoelectron spectroscopy (XPS) using an ESCALAB MKII spectrometer (VG Co., United Kingdom).

2.4. Assessment of photothermal effects

The rise in temperature of GO, Pd NPs, and GO-Pd NPs following NIR (3.0 W/cm^2) laser irradiation was evaluated for up to 5 min. Images for the temperature change were recorded using a thermal camera (Therm-App TH, Isreal). Different concentrations (0.0, 1.0, 2.5, 5.0, 7.5,