Gold Nanoshells With Micellar Core for The Delivery of Hydrophobic Chemotherapeutic and Cancer Photothermal Therapy

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ABSTRACT

In the present study, we established a multifunctional gold nanoshell (GNS) that utilizing positively charged PDMA-b-PCL micelles as a template. By growing the gold layer on the surface of micelles, this newly synthesized GNS with micellar core enable the delivery of hydrophobic chemotherapeutics. The synthesized SN-38@pGNS had a spherical morphology and a narrow size distribution. With the exposure to NIR laser irradiation, SN-38@pGNS exhibited an excellent photothermal conversion efficiency. Moreover, the combination of chemotherapy and photothermal therapy resulted in a significant anticancer effect in the *in vitro* study. These results suggested the gold nanoshells with micellar core can serve as not only a photothermal mediator but also a delivery system for hydrophobic drugs. Therefore, the synthesized gold nanoshells with micellar core can be applied as a potential multifunctional therapeutics for cancer therapy in the future.

Keywords: gold nanoshell, micelles, SN-38, chemotherapy, photothermal therapy

1 INTRODUCTION

Cancer therapy is one of the robust topics for biomedical research nowadays. The development of multifunctional nanomedicine has been an intensively explored fields, since it could optimize the safety and efficacy of therapeutic regimens owing to the combination of several useful properties such as longevity in blood, specific targetability, controlled release and contrast or drug loading. Among the metallic nanoscaled cancer diagnostics and therapeutics, gold nanoparticles have been studied extensively due to their well-established biocompatibility, easy preparation methods, and high stability [1]. Gold nanoparticles possess optical properties such as strong absorption and scattering in the visible–near-infrared (VIS–NIR) region depending on their size and shape. Under the light exposure, the conduction band electrons of the gold nanoparticles are polarized by the oscillating electro-magnetic field of the light, and undergo a collective coherent resonant oscillation. At a specific frequency of the incident light, this oscillation is resonant and is called surface plasmon resonance (SPR). The oscillated electrons interact with phonons by the electron-phonon and phonon-phonon interactions, exchanging the energy to the gold lattice, and then transfer the heat to the surrounding environment, resulting in heat generation in local area. This characteristic makes gold nanoparticles an excellent photo-thermal mediator [2].

In the past decade, gold nanoshells (GNS) have attracted great research interest in biomedical applications. Because of their stable structure, tunable resonances, and the good photothermal conversion efficiency, GNS could be utilized in both diagnostic and therapeutic applications, such as drug delivery, phothermal cancer therapy, and biomedical imaging probes [3–6]. Solid or hollow silica nanospheres [6,7], polystyrene nanospheres [8], and poly(lactic-co- glycolic acid) (PLGA) nanospheres [9] were reported as the template for the core-shell formation in previous studies. In the present study, we utilized the positively charged PDMA-b-PCL micelles as a template for GNS formation. The established GNS not only enable the delivery of the hydrophobic chemotherapeutic, SN-38, but also exhibit excellent photothermal conversion ability. Therefore, with the combination of chemotherapy and photothermal therapy, the gold nanoshells with micellar core have great potential to be applied as a potential multifunctional therapeutics for cancer therapy.

2 MATERIALS AND METHODS

2.1 Synthesis of the Micellar Core and Gold Nanoshells

The amphiphilic PDMA-block-PCL (PDMA-b-PCL) diblock copolymers were synthesized using the methods we described previously [10]. The Poly(2-(dimethylamino) ethyl methacrylate) (PDMA-OH) was first synthesized via the free-radical polymerization of 2-(dimethylamino)ethyl methacrylate (DMAEMA) monomers. The diblock PDMA-b-PCL copolymers were then prepared by the following ring-opening polymerization of ε-caprolactone, using stannous octoate $(SnOct)_2$ as the catalyst. The PDMA-b-PCL powder was collected by precipitation in diethyl ether and was dried at room temperature under vacuum.

PDMA-b-PCL micelles and SN-38-encapsulated micelles (SN-38@M) were prepared via the lyophilization-hydration method. For SN-38@M, SN-38 (7-ethyl-10-hydroxycamptothecin) in dimethyl sulfoxide (DMSO) and PDMA-b-PCL in tetrahydrofuran (THF) were mixed together at a drug/polymer weight ratio (D/P ratio) of 1/20, and gently agitatd until the formation of a clear solution. The mixture was then lyophilized for 24 hours. The lyophilized cake was rehydrated in deionized water at 70 °C, followed by the sonication using an ultrasonic cell crusher for further dispersion of the micelles. Afterward, the micelles solutions were filtered through a 0.45-mm cellulose acetate filter membrane and stored at 4 °C before use.

The synthesis the gold nanoshells with the micellar core was modified from the previously reported methods [4,7]. In brief, 4 μM chloroauric acid ($HAuCl_4$, pH 6.5) and 2 μM hydroxylamine (NH_2OH) was added into PDMA-b-PCL micelles or SN-38@M while stirring to deposit gold seeds on the micellar surface. After reacting for 15 min, 40 μM $HAuCl_4$ (pH 6.5) was added into the solution, and 20 μM NH_2OH was added dropwise to form the gold nanoshell. The mixture was then stirred for another 30 min. The synthesized gold nanoshells were centrifuged (18000 g, 7 min) and washed with deionized water for three times. The gold nanoshells were further pegylated by thio-terminated methoxypoly(ethylene glycol) ($mPEG_{5000}$-SH) and purified by centrifugation.

2.2 Characterizations of the Micellar Core and Gold Nanoshells

A Zetasizer Nano-ZS90 (Malvern Instruments Ltd., Worcestershire, UK) was used to measure the particle size, polydispersity index (PDI), and zeta potential of synthesized micelles and gold nanoshells by dynamic light scattering (DLS) and laser Doppler electrophoresis. The morphology of the micelles and gold nanoshells was observed by the transmission electron microscopy (TEM; HITACHI H-7500, Tokyo, Japan). A drop of sample solution was placed on a carbon-coated copper grid. After removal of the excess solution, the micellar sample was negatively stained with 1% (w/v) uranyl acetate (UA).

The amount of SN-38 in SN-38@M and SN-38@GNS were measured by high performance liquid chromatography (HPLC) [11]. Standard samples with known concentrations of SN-38 in DMSO were prepared, and SN-38@M and SN-38@GNS were also dissolved and diluted in DMSO. These prepared samples were passed through 0.2-mm PVDF microfilters and was injected into the HPLC system. Separation was achieved on a Waters Symmetry® C_{18} reversed-phase column (150 mm × 3.9 mm, 5 μm), and the mobile phase consisted of 0.1% trifluoroacetic acid and acetonitrile [60:40 (v/v)] at a flow rate of 1 mL/min. SN-38 was detected by fluorescence with the excitation/emission wavelengths of 365/550 nm.

2.3 Photothermal Conversion Efficiency

First, the absorption spectra of synthesized pGNS and SN-38@pGNS were assessed by using a spectrometer (SpectraMax M2 Multimode Microplate Reader, Molecular Devices, USA). The temperature profiles of synthesized pGNS and SN-38@pGNS during NIR irradiation were analyzed in a 96-well plate with thermocouple needles. 100 μL of sample solutions were added into wells, and each well was irradiated by the NIR laser (808 nm) at a power density of 1.9 W/cm^2 for 10 min. The temperature was recorded continuously during the irradiation.

2.4 *In Vitro* Cytotoxicity and Anticancer Effect

A human colon cancer cell line, HCT116, was cultured in McCoy's 5a modified medium (GIBCO®) supplemented with 10% fatal bovine serum (FBS) and 1% penicillin-streptomycin-amphotericin at 37 °C in an atmosphere of 5% CO_2. The *in vitro* anticancer effects of SN-38@M, pGNS, SN-38@pGNS and the combined photothermo-chemotherapy were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. HCT116 cells were seeded in 96-well plates at a density of 10^4 per well and incubated overnight. Next, the cells were treated with different synthesized reagents for 24 hours, including pGNS, SN-38@GNS, and SN-38@M (the concentration of SN-38 was 0.26 μM for treatments contained chemo-therapeutics). And after that, laser-treated groups received the NIR laser irradiation (808 nm, 1.9 W/cm^2 for 5 or 10 min). All groups were incubated for another 24 hours, and then the medium was replaced with fresh serum-free medium containing 0.5 mg/mL MTT, and the cells were incubated for another 3 hours. Finally, the medium was removed, and DMSO was added to each well to dissolve the formazan crystals. The absorbance at 570 nm was measured by an ELISA reader and the cell viability of each group was expressed as a percentage relative to that of the control group.

2.5 Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). The significance of differences between groups in this study was analyzed using Student's t-test. A value of $P < 0.05$ was considered statistically significant.

3 RESULTS

The PDMA-b-PCL micelles with positive surface charge were synthesized as previously reported [10]. After synthesis of PDMA-b-PCL copolymers via the free-radical polymerization and ring-opening polymerization, self-assembled PDMA-b-PCL micelles were prepared by the lyophilization hydration method and the chemotherapeutic

SN-38 was encapsulated into the micellar core at a drug/polymer (D/P) ratio of 1/20. The average hydrodynamic diameter of PDMA-b-PCL micelles was 93.4±0.96 nm, and that of SN-38-encapsulated micelles (SN-38@M) was 121.3±1.46 nm. The encapsulation efficiency of SN-38 was 46.7±9.16%. Both PDMA-b-PCL micelles and SN-38@M possessed positive surface charge (around 32 ~ 35 mV, Table 1), which can attract negatively charged $AuCl_4^-$. And then $AuCl_4^-$ was reduced by the reducing agent and forming a gold nanoshell on the micellar surface. The size of synthesized gold nanoshells (GNS) increased to 167.1±2.40 nm due to the successful coating of gold layers on the micellar core. Similarily, the size of SN-38-loaded GNS (SN-38@GNS) was also larger than that of SN-38@M (Table 1).

The transmission electron microscope (TEM) image also depicted the spherical morphology and uniform size distribution of SN-38@GNS (Fig. 1). And it is noted that the size of SN-38@M observed under TEM was smaller than the hydrodynamic diameter measured by the zeta sizer, because of the shrinkage resulted from the dehydration during sample preparation for TEM. The empty GNS and SN-38@GNS were then functionalized with thio-polyethylene glycol (PEG-SH, Mw = 5 kDa) to improve their biocompatibility and prolong the *in vivo* circulation time for the future application.

The absorbance spectra of pGNS and SN-38@pGNS both revealed a broad absorption band between 500 and 1000 nm (Fig. 2). The strong absorption in the near-infrared (NIR) region allows GNS to be a potential photothermal mediator for cancer therapy. To investigate the photothermal conversion efficiency of GNS, we measured

	Diameter (nm)	PDI	Zeta potential (mV)
Empty micelles	93.4 ± 0.96	0.190	35.1 ± 1.08
Empty GNS	167.1±2.40	0.123	45.1 ± 0.76
SN-38@M	121.3±1.46	0.201	32.7 ± 0.55
SN-38@GNS	131.1±1.60	0.170	45.5 ± 1.28

Table 1. Sizes and zeta potentials of synthesized drug-free micelles, GNS and SN-38-loaded micelles and GNS.

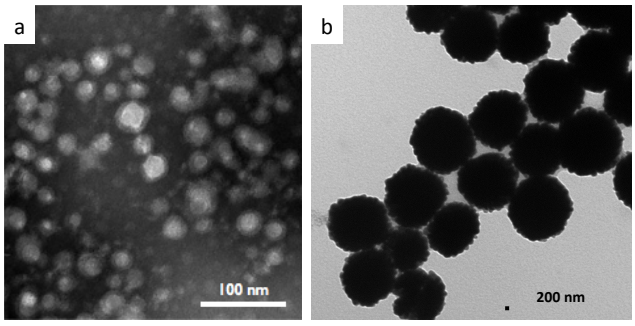

Fig. 1. Transmission electron micrographs of (a) SN-38@M and (b) SN-38@GNS.

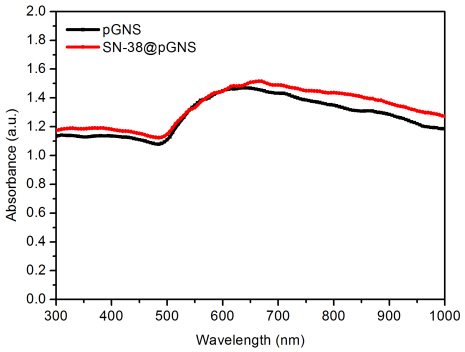

Fig. 2. Ultraviolet-visible absorption spectra of pegylated GNS (pGNS) and SN-38@pGNS.

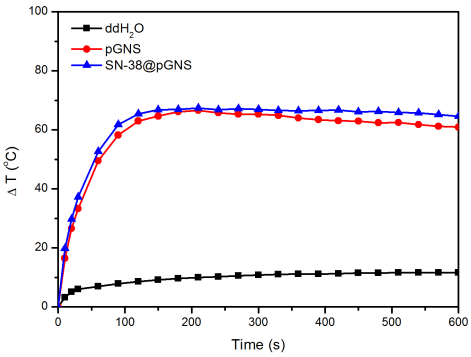

Fig. 3. Temperature profiles of pGNS and SN-38@pGNS under the NIR irradiation (808 nm, 1.9 W/cm^2 for 10 min).

the temperature changes of GNS solutions under the NIR laser irradiation (808 nm, 1.9 W/cm^2 for 10 min). As shown in Fig. 3, both pGNS and SN-38@pGNS exhibited excellent photothermal conversion ability, with a temperature increase of 60°C within 2 min of irradiation.

We used MTT assay to study the therapeutic effect of the synthesised multifunctional GNS in a human colon cancer cell line, HCT116. HCT116 cells were treated with different synthesized reagents (the concentration of SN-38 was 0.26 μM for treatments contained chemotherapeutics) for 24 hours, and after that, laser-treated groups received the NIR laser irradiation (808 nm, 1.9 W/cm2 for 5 or 10 min). All groups were incubated for another 24 hours, and then the cell viability was evaluated. The NIR irradiation itself did not significantly decreased the cell viability (Fig. 4), indicating the laser power density and irradiation time were safe for HCT116. And the pGNS-treated group had more than 70% of cells remained viable, suggesting an acceptable level of biocompatibility of the empty pGNS. While the photothermal therapy (pGNS plus laser irradiation) only showed limited therapeutic effect, the combined phothermo-chemotherapy involving SN-38@pGNS and NIR laser irradiation (10 min) resulted in a significant anticancer effect.

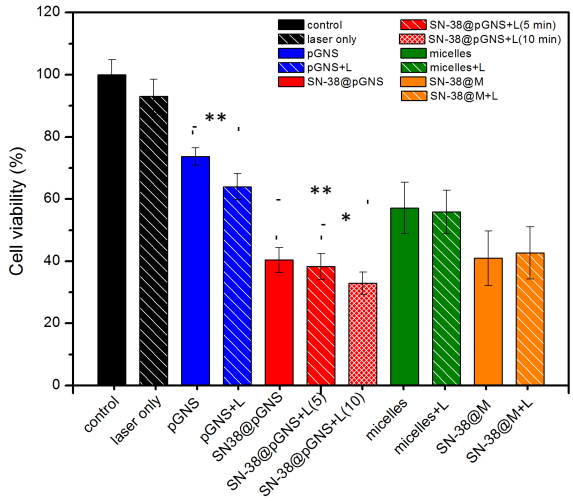

Fig. 4. The evaluation of the in vitro anticancer effect of the synthesized multifunctional GNS in HCT116 cells. (**: P<0.01, *: P<0.05.)

4 CONCLUSION

We established a multifunctional gold nanoshell (GNS) by utilizing positively charged PDMA-b-PCL micelles as the template. The GNS with micellar core enable the delivery of the hydrophobic chemotherapeutic, SN-38. The synthesized SN-38@pGNS had a spherical morphology and a narrow size distribution. With the exposure to NIR laser irradiation, SN-38@pGNS exhibited an excellent photothermal conversion efficiency. And as shown in the *in vitro* study, The combination of chemotherapy and photothermal therapy resulted in a significant anticancer effect. These results suggested the gold nanoshells with micellar core can serve not only as a photothermal mediator but also a delivery system for hydrophobic drugs. Therefore, the gold nanoshells with PDMA-b-PCL micellar core we reported here could be applied as a potential multifunctional therapeutics for cancer therapy in the future.

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