Hollow Nanostars with Photothermal Gold Caps and Their Controlled Surface Functionalization for Complementary Therapies

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Gold nanoparticles exhibiting absorption in the desirable near-infrared region are attractive candidates for photothermal therapy (PTT). Furthermore, the construction of one nanoplatform employing gold nanoparticles for complementary therapy is still a great challenge. Here, well-defined unique hollow silica nanostars with encapsulated gold caps (starlike Au@SiO₂) are readily synthesized using a sacrificial template method. Ethanolamine-functionalized poly(glycidyl methacrylate) (denoted as BUCT-PGEA) brushes are then grafted controllably from the surface of starlike Au@SiO₂ nanoparticles via surface-initiated atom transfer radical polymerization to produce starlike Au@SiO₂-PGEA. The photothermal effect of gold caps with a cross cavity can be utilized for PTT. The interior hollow feature of starlike Au@SiO₂ nanoparticles endows them with excellent drug loading capability for chemotherapy, while the polycationic BUCT-PGEA brushes on the surface provide good transfection performances for gene therapy, which will overcome the penetration depth limitation of PTT for tumor therapy. Compared with ordinary spherical Au@SiO₂-PGEA counterparts, the starlike Au@SiO₂-PGEA hybrids with sharp horns favor endocytosis, which can contribute to enhanced antitumor effectiveness. The rational integration of photothermal gold caps, hollow nanostars, and polycations through the facile strategy might offer a promising avenue for complementary cancer therapy.

1. Introduction

Gold nanoparticles have stimulated tremendous efforts due to their inherent attributes such as biocompatibility, stability, and tunable optical properties.[3] In particular, the strong optical absorption resulting from surface plasmon resonance (SPR) is appealing, while the absorbed energy could be converted into heat by the Landau damping effect.[2] Gold nanoparticles exhibiting absorption in the desirable near-infrared (NIR) region are supposed to be attractive candidates for photothermal therapy (PTT) since the attenuation of light by blood and soft tissue is relatively low.[3,4] PTT has attracted intensive attention for cancer therapy due to the outstanding advantages such as low cost and minimal side effects.[5–8] However, PTT is not effective enough for the treatment of tumors located in deep sites due to limited penetration depth.[9,10] As a supplementary therapy modality, chemotherapy could overcome this drawback. Gene therapy (GT) provides a promising way to deliver specific genes into targeted cells to restore defective genes or promote specific cellular functions, which could realize long-term treatment.[11] Although combined cancer treatment could be realized by multimodal therapy,[12–14] the construction of one nanoplatform employing gold nanoparticles to integrate PTT, GT, and chemotherapy ingeniously in one system is still a great challenge. Physicochemical features including size, shape, surface functionalization, and dispersion state of nanoparticles would all affect their performances.[15,16] Gold nanocaps or semishells demonstrate novel plasmonic and optical properties due to the presence of unique cavities within gold nanoparticles.[17–19] Redshifted optical absorption in contrast with ordinary solid spherical nanoparticles renders them promising photothermal conversion agents.[18,20] It is noticeable that gold caps with a sophisticated cross-shaped cavity were fabricated by controlled etching of the PbS-Au nanostar–nanoparticle heterodimers.[21] However, considerable aggregation and clustering of gold caps in solution prevent investigation of their optical properties and further biomedical applications. Hollow silica nanoparticles with the characteristics of interior cavity, favorable large specific surface areas, and facile surface functionalization are supposed to be prominent as drug carriers for chemotherapy.[22–25] In our recent work, special starlike hollow silica carriers with six symmetrical sharp horns were demonstrated superior abilities for cell endocytosis and drug/gene codelivery.[26] It could be imaged that well-dispersed starlike hollow carriers comprising unique gold caps will be desirable for cancer therapy.

Herein, we synthesized novel starlike hetero-nanostructures (starlike Au@SiO₂) where gold caps were encapsulated in...
hollow silica nanostars. Sharing the identical star-shaped PbS nanoparticle as the sacrificial template, the fixed gold nanocap and hollow silica nanostar were integrated in one nanostructure (Figure 1). Meanwhile, the silica coating guarantees high dispersibility of gold nanocaps in aqueous solution to achieve photothermal effects. The resultant unique starlike Au@SiO$_2$ with six sharp horns was found to favor cell endocytosis and drug loading. In order to realize efficient gene therapy, low toxic hydroxyl-rich ethanolamine (EA)-functionalized poly(glycidyl methacrylate) (PGMA) (BUCT-PGEA)\textsuperscript{[26–29]} was proposed to functionalize starlike Au@SiO$_2$ via surface-initiated atom transfer radical polymerization (ATRP) to produce starlike Au@SiO$_2$-PGEA. Thus, the exterior cationic surfaces of the starlike Au@SiO$_2$-PGEA nanohybrids could be utilized to condense DNA and the interior cavity for anticancer drug loading. Meanwhile, the encapsulated gold caps could result in PTT for complementary therapy of GT and chemotherapy in the same nanostructure. Furthermore, the cap-like gold nanoparticles could be used for photoacoustic (PA) and computer tomography (CT) imaging which could realize imaging-guided cancer therapy. The effects of morphology on the therapeutic efficacy were also investigated by fabricating ordinary spherical Au@SiO$_2$-PGEA nanohybrids as the counterparts.

2. Results and Discussion

2.1. Synthesis and Characterization of Au@SiO$_2$ Nanoparticles

The starlike Au@SiO$_2$ hetero-nanoparticles with encapsulated gold caps were prepared employing star-shaped PbS nanoparticles as sacrificial templates (Figure 1). First, cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate were explored as capping agents to prepare star-shaped PbS nanoparticles with the size of 80 nm in aqueous solution.\textsuperscript{[30]} As shown in Figure 2a, well-defined star-shaped PbS nanoparticles with six symmetrical horns were monodisperse. Then, the PbS-Au nanostructure with a gold nanoparticle on one horn was synthesized by reducing HAuCl$_4$ with ascorbic acid according to the procedure described elsewhere,\textsuperscript{[21]} as displayed in Figure 2b,b'\textsuperscript{.} Thereafter, PbS-Au@SiO$_2$ core–shell nanoparticles were prepared by coating a mesoporous silica layer on the surface of PbS-Au nanoparticles through controlled hydrolysis and condensation of tetraethylorthosilicate (TEOS) in the presence of CTAB.\textsuperscript{[31]} As shown in Figure 2c,e, starlike and spherical PbS-Au@SiO$_2$ hetero-nanostructures were produced by adjusting the concentration of TEOS to produce silica layers with different thicknesses. Finally, PbS templates were removed exhaustively by etching with HCl. The resultant Au@SiO$_2$ nanochips are shown in Figure 2d,f. Starlike and spherical Au@SiO$_2$ nanohybrids possess the same star-shaped cavity, which is the duplicate of the star-shaped template. As a result, well-dispersed starlike and spherical Au@SiO$_2$ nanohybrids with comparable over-size of \approx$120$ nm and encapsulated Au nanocaps of \approx$40$ nm were successfully fabricated. Different from the rattle structure, Au nanocaps are unmovable in the silica nanostars.

2.2. Synthesis and Characterization of Au@SiO$_2$-PGEA Nanohybrids

Four types of Au@SiO$_2$-PGEA nanohybrids were prepared by grafting cationic BUCT-PGEA brushes from the surface of
Au@SiO₂ nanoparticles via surface-initiated ATRP (Figure 1). To immobilize the ATRP initiator, Au@SiO₂-NH₂ was first produced through surface modification with 3-aminopropyltriethoxysilane. Then, the bromoisobutyryl group-terminated Au@SiO₂ (Au@SiO₂-Br) with initiation sites was synthesized by the reaction of Au@SiO₂-NH₂ with 2-bromoisobutyryl bromide (BIBB). Well-defined Au@SiO₂-PGMA nanohybrids were subsequently prepared using Au@SiO₂-Br via ATRP of GMA. Finally, Au@SiO₂-PGEA was synthesized through ring-opening reaction between epoxy groups of Au@SiO₂-PGMA and excess EA. Two types of Au@SiO₂-PGEA nanohybrids with different lengths of BUCT-PGEA brushes were obtained through controlling the amounts of GMA and ATRP reaction time. The functionalization processes were verified by X-ray photoelectron spectroscopy (Figure S1, Supporting Information), while the weight ratio of BUCT-PGEA brushes was determined by thermal gravimetric analysis (Figure S2, Supporting Information). The redshift of ≈10 nm in the absorption spectra of Au@SiO₂-PGEA nanohybrids compared with Au@SiO₂, which corresponds to the refractive index change also confirmed the presence of polymer coating (Figure S3a, Supporting Information). In this work, we fabricated four types of Au@SiO₂-PGEA nanohybrids with different morphologies of Au@SiO₂ nanoparticles and weight ratios of BUCT-PGEA (~40 and ~80 wt%), namely starlike Au@SiO₂-PGEA1, starlike Au@SiO₂-PGEA2, spherical Au@SiO₂-PGEA1, and spherical Au@SiO₂-PGEA2, respectively.

2.3. Optical Properties and Photothermal Effect of Au@SiO₂-PGEA Nanohybrids

The SPR absorption of starlike Au@SiO₂-PGEA nanohybrids was recorded by UV–vis spectroscopy (Figure S3a, Supporting Information). A broad SPR band in the NIR region was observed resulting from the unique morphology of the encapsulated gold nanocaps. In contrast, the solid gold nanoparticles with comparable size of 41 nm showed barely any absorption in the NIR region (Figure S3a,b, Supporting Information). The absorption of starlike Au@SiO₂-PGEA nanohybrids in the desirable NIR region drove us to investigate their photothermal performance. The temperature variation of solutions with different concentrations was monitored upon NIR laser irradiation. As shown in Figure 2g, the temperature of starlike...
Au@SiO₂-PGEA2 suspension evidently increased after 10 min irradiation, while the temperature of water hardly rose under the same condition. Furthermore, the concentration of starlike Au@SiO₂-PGEA2 was observed to affect the temperature increase. For example, the temperature of suspension with the concentration of 0.3 mg mL⁻¹ increased from 22 to 50 °C, while the temperature rose to 60 °C at the concentration of 2.4 mg mL⁻¹ over a 10 min irradiation. Moreover, we investigated the photothermal performance of starlike Au@SiO₂-PGEA2 in medium with different ionic strengths. As shown in Figure S4 (Supporting Information), starlike Au@SiO₂-PGEA2 exhibited comparable heating efficiency in NaCl solutions with different concentrations including saline, which possesses similar colloid osmotic pressure to blood plasma.

The excellent photothermal effect of the starlike Au@SiO₂-PGEA nanohybrids in vitro further inspired us to examine the in vivo performance. The infrared thermal imaging and temperature variation of the tumor areas in the presence of NIR laser at different time points was monitored by an IR thermal camera. As shown in Figure 2h, the tumor treated with phosphate buffered saline (PBS) exhibited barely any temperature change after laser irradiation, while the tumor with the injection of starlike Au@SiO₂-PGEA2 exhibited remarkable color change, which is in agreement with the results of temperature variation as displayed in Figure 2i. The temperature of the tumors reached ~46 °C after 10 min irradiation, demonstrating the superior photothermal performance of starlike Au@SiO₂-PGEA in vivo. These results above demonstrated the feasibility of starlike Au@SiO₂-PGEA nanohybrids to result in environmental heat as photothermal agents.

2.4. Gene Delivery Capability of Au@SiO₂-PGEA Nanohybrids In Vitro

For efficient gene carriers, pDNA condensation capability is of great significance. The relative amount of Au@SiO₂-PGEA to pDNA was defined as N/P ratio, which is calculated from the molar ratio of nitrogen (N) in Au@SiO₂-PGEA to phosphate (P) in pDNA. The electrophoretic mobility of Au@SiO₂-PGEA/pDNA complexes at various N/P ratios was first analyzed. The pDNA retardation within the N/P ratio of 1.5 confirmed the good capability of Au@SiO₂-PGEA to compact pDNA (Figure 3a).

The complexes of Au@SiO₂-PGEA/pDNA were also required to possess appropriate sizes and surface charges to facilitate cellular uptake. Dynamic light scattering measurements were carried out to obtain the hydrodynamic particle sizes and surface potentials of complexes. As displayed in Figure 3b, all Au@SiO₂-PGEA/pDNA complexes showed smaller sizes (within 300 nm) compared with pristine nanohybrids. The zeta potentials of all Au@SiO₂-PGEA/pDNA complexes at various N/P ratios are positive to favor cellular internalization (Figure 3c). The morphologies of Au@SiO₂, Au@SiO₂-PGEA2, and Au@SiO₂-PGEA2/pDNA (N/P = 15) were further characterized by atomic force microscopy (AFM) imaging (Figure 3d–f). The AFM image of starlike Au@SiO₂ nanoparticles clearly revealed the starlike morphology with six symmetric horns and a nanoparticle on one horn (Figure 3d). After surface functionalization, the size of particles increased substantially from ~130 to ~215 nm, while the morphology of nanohybrids was observed to be quasispherical probably due to the coating of BUCT-PGEA brushes (Figure 3e). As shown in Figure 3f, the AFM image of Au@SiO₂-PGEA2/pDNA complexes exhibits the morphology of heterostructures while the size decreased slightly to be ~180 nm, reflecting the shrinkage of the polymer brushes. The AFM imaging results along with particle size and zeta potential measurement results further confirmed the DNA-condensing ability of Au@SiO₂-PGEA nanohybrids (Figure 3b,c). The stability of the Au@SiO₂-PGEA/pDNA complexes was further investigated by evaluating the particle size of complexes in media with different ionic strengths. As shown in Figure S5 (Supporting Information), starlike Au@SiO₂-PGEA2/pDNA (N/P = 15) show similar size in NaCl solutions with different concentrations, suggesting the excellent stability of complexes in saline.

It is also essential to assess the cytotoxicity of Au@SiO₂-PGEA carriers before their utilization for gene delivery. The cell viability of the Au@SiO₂-PGEA/pDNA complexes was evaluated employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in the selected C6 and HEK293 cell lines. As displayed in Figure 4a, at the concentration of Au@SiO₂-PGEA nanohybrids (or N/P ratio) increasing, the cell viability of the four types of complexes fabricated from starlike and spherical Au@SiO₂ nanoparticles gradually decreased, which might be caused by the existence of free cationic nanohybrids besides the compact complexes at higher N/P ratios. It is worth noting that compared with the “gold-standard” branched polyethylenimine (PEI, M₀ ~25 kDa), all the Au@SiO₂-PGEA nanohybrids exhibited lower cytotoxicity at various N/P ratios in both C6 and HEK293 cell lines.

Luciferase was first employed as the reporter gene to investigate the gene transfection efficiency of Au@SiO₂-PGEA/pDNA complexes in both C6 and HEK293 cell lines. As shown in Figure 4b, at the N/P ratios of 15 or higher, all Au@SiO₂-PGEA nanohybrids showed higher transfection efficiencies than PEI (25 kDa) at its optimal N/P ratio of 10, verifying the potential of Au@SiO₂-PGEA as prominent gene carriers. The transfection efficiencies of Au@SiO₂-PGEA were observed to first increase and then decrease with the N/P ratio. It implies that at the optimal N/P ratio of 15 or 20, appropriate amount of Au@SiO₂-PGEA nanohybrids was adequate to condense pDNA while negligible cytotoxicity might be caused by nanohybrids (Figure 4a). In addition, compared with Au@SiO₂-PGEA1/pDNA complexes, Au@SiO₂-PGEA2/pDNA complexes showed higher transfection efficiencies in both cell lines, indicating that the weight ratio of the coating polymer affects the gene delivery capability of Au@SiO₂-PGEA nanohybrids.[24]

It is worth noting that starlike Au@SiO₂-PGEA carriers performed better than the spherical analogues, confirming that the morphology of nanohybrids matters much in gene transfection. This observation is also consistent with our previous work that starlike SiO₂-PGEA nanohybrids with sharp horns could facilitate transfection compared to spherical ones.[26] In C6 cell lines, direct visualization of enhanced green fluorescent protein (EGFP) gene expression was also carried out to further verify the gene transfection performances of Au@SiO₂-PGEA2. Representative fluorescence images of EGFP gene expression
mediated by Au@SiO2-PGEA2 at the optimal N/P ratio of 15 are shown in Figure 4c. Starlike Au@SiO2-PGEA2 and spherical Au@SiO2-PGEA2 represented the percentage of 25% and 32% of EGFP-positive cells, respectively while PEI displayed the much lower percentage of 14%, confirming the superiority of starlike carriers.

2.5. Cellular Internalization

To clarify the correlation of cellular uptake and gene transfection, the cellular internalization of Au@SiO2-PGEA2/pDNA complexes was analyzed in C6 cell lines. As shown in Figure 4d, the C6 cells treated with starlike Au@SiO2-PGEA2/pDNA complexes showed much stronger fluorescence signals of YoYo-1 labeled pDNA than the spherical counterparts. Flow cytometry was used to analyze the cellular internalization of the corresponding complexes (Figure 4d). Starlike Au@SiO2-PGEA2/pDNA complexes exhibited the cellular internalization ratio of 87.7%, while the spherical complexes showed relatively lower ratio of 76.2%. These results revealed that the morphology of Au@SiO2-PGEA nanohybrids affects their cellular uptake and the resultant gene transfection. Starlike Au@SiO2-PGEA2 with higher cellular internalization could contribute to superior gene transfection. Thus, starlike Au@SiO2-PGEA2 was elected as the model platform for the following complementary therapy.

2.6. NIR- and pH-Responsive Drug Release

Since the interior cavity of starlike Au@SiO2-PGEA2 was suitable for drug loading, it would be ideal to achieve NIR-responsive drug release through photothermal effect. The photothermal effect of starlike Au@SiO2-PGEA2 nanohybrids was first evaluated in C6 cell line. After being treated with starlike Au@SiO2-PGEA2 under NIR irradiation for 10 min, the live/dead C6 cells displayed in green/red fluorescence was visualized by double staining with fluorescein diacetate (FDA) and propidium iodide (PI). As shown in Figure 5a,b, ~60% of cell death was observed when the cells were treated with starlike Au@SiO2-PGEA2 suspensions under irradiation while the control cells remained unchanged in the entire region without
Au@SiO₂-PGEA2. Thereafter, anticancer drug DOX was loaded in the cavity of starlike nanohybrids to form DOX@starlike Au@SiO₂-PGEA2 with the loading content of 5.0% and entrapment efficiency of 40%. The encapsulated DOX was monitored by the absorption of DOX@starlike Au@SiO₂-PGEA2 dispersion at 485 nm.

We investigated the drug release behavior of DOX@starlike Au@SiO₂-PGEA2 at different pH values. As shown in Figure S6 (Supporting Information), most DOX was kept inside the cavity and only about 5% drug was released at pH = 7.4 within 240 min, while about 20% drug was released in acidic environment (pH = 5.6). The pH-triggered DOX release might result from the protonation of cationic polymer coatings, which facilitates modest escape of DOX from the orifices on the surface of silica. The pH responsiveness is supposed to benefit the anticancer drug releasing due to the acidic tumor microenvironment. Based on the photothermal effect of starlike Au@SiO₂-PGEA2 nanohybrids, the NIR-responsiveness of DOX release was further investigated under acidic condition with interval NIR irradiation. When the NIR radiation at
a power density of 2.0 W cm$^{-2}$ was applied for 10 min, obvious increase in drug release was displayed (Figure S6, Supporting Information). When the NIR irradiation was switched off, the drug release slowed down obviously. These results indicate that NIR irradiation could successfully accelerate the drug release of DOX@starlike Au@SiO$_2$-PGEA2.

The antitumor effect of DOX@starlike Au@SiO$_2$-PGEA2 was then investigated. MTT assay was employed to evaluate the cell viabilities of glioma C6 cells treated with free DOX and DOX@starlike Au@SiO$_2$-PGEA2/pDNA in the absence and presence of NIR irradiation. As shown in Figure 5b, free DOX at the amount equivalent to that loaded in DOX@starlike Au@SiO$_2$-PGEA2 show moderate cytotoxicity and the cell viability was about 65%. In contrast, the cell viability treated with DOX@starlike Au@SiO$_2$-PGEA2/pDNA was relatively high ($\approx$ 85%), suggesting that the drug release was blocked by the condensed polymer chains on the surface of silica (Figure S6, Supporting Information). The antitumor ability of DOX@starlike Au@SiO$_2$-PGEA2 was suppressed. However, when NIR irradiation was applied, considerable decrease in the cell viability was observed ($\approx$ 30%), where the photothermal effect of gold nanocaps and NIR-responsive drug release from the cavity contributed to tumor cell death.

The intracellular trafficking behaviors of DOX@starlike Au@SiO$_2$-PGEA2/pDNA were further monitored by confocal laser scanning microscopy. During the process, the fluorescence of DOX in red and DAPI labeled nucleus in blue could be visualized in C6 cells under excitation. As shown in Figure 5c, after 4 h incubation, the cells mediated by DOX@starlike Au@SiO$_2$-PGEA2/pDNA without NIR irradiation maintained the normal spindle morphology (Figure S7a, Supporting Information) and DOX distributed all around the cell cytoplasm. When irradiated by NIR laser, the C6 cells treated with DOX@starlike Au@SiO$_2$-PGEA2/pDNA showed noticeable morphological evolution to round shape (Figure S7b, Supporting Information), indicating the efficient cell apoptosis. The highly overlapped fluorescence signals originated from DOX and DAPI suggest that the released DOX entered nucleus. These phenomena confirmed the NIR-responsive drug release from DOX@starlike Au@SiO$_2$-PGEA2/pDNA complexes to kill tumor cells substantially through combination of drug delivery and photothermal effect.

2.7. Complementary PTT/GT/Chemotherapy In Vitro and In Vivo

With the expectation of complementary antitumor effect through combining different therapeutic modalities, we first evaluated the efficacy of PTT, GT, and chemotherapy with a preliminary experiment in vitro. A promising tumor-suppressor antioncogene p53, which is commonly used in clinical trials, was employed to inhibit tumor cell proliferation.$^{13}$ The C6 cells were incubated with starlike Au@SiO$_2$-PGEA2/p53 complexes,
which were speculated to effectively deliver plasmid p53 (cloned with p53 gene) to C6 cells for gene therapy (Figure 4b) with compromised cytotoxicity (Figure 4a). As shown in Figure S8 (Supporting Information), compared with naked p53, the significant decrease in cell viability mediated by starlike Au@SiO2-PGEA2/p53 from ≈90% to ≈50% suggests the great potential of starlike Au@SiO2-PGEA2 as carriers for gene therapy. Among the groups of starlike Au@SiO2-PGEA2/p53, starlike Au@SiO2-PGEA2/p53+NIR, and DOX@starlike Au@SiO2-PGEA2/p53+NIR, the combination of triple modality therapy exhibited the lowest cell viability of ≈30%, demonstrating the prominent effect of complementary therapy by employing DOX@starlike Au@SiO2-PGEA2/p53.

Motivated by the good results of combined GT/PTT/chemotherapy in vitro, the in vivo antitumor performance of DOX@starlike Au@SiO2-PGEA2/p53 was further investigated. The glioma tumor-bearing mice were randomly divided into six groups with different treatments: PBS group (G1, control), starlike Au@SiO2-PGEA2/p53 group (G2, GT), DOX@starlike Au@SiO2-PGEA2 group (G3, chemotherapy), starlike Au@SiO2-PGEA2+NIR group (G4, PTT), DOX@starlike Au@SiO2-PGEA2+NIR group (G5, PTT/chemotherapy), and DOX@starlike Au@SiO2-PGEA2/p53+NIR group (G6, PTT/GT/chemotherapy). Intratumoral administration was performed following the routine treatment of glioma to avoid the blood–brain barrier.[34] The administration was carried out five times for 12 d except that the PTT group was only injected once in order to minimize detrimental side effect. The NIR laser irradiation in PTT G4, G5, and G6 groups was performed by an 808 nm laser only once after the first injection. The length and width of the tumors were measured during the 12 d administration and the relative tumor volume was recorded as a function of time. After the treatment, all mice were euthanized and the tumors were imaged (Figure 6a) and weighed (Figure S9, Supporting Information). The tumors in the control G1 group grew rapidly during the 12 d. In the G2 group, tumors were apparently suppressed compared to the control group, consistent with the good transfection performance of starlike Au@SiO2-PGEA2 (Figure 4b) and excellent antitumor ability of p53 in gene therapy in vitro (Figure S8, Supporting Information). There was obvious tumor growth inhibition to some extent in the G3 and G4 groups (Figure 6a), indicating the efficacy of single-modal chemotherapy and PTT. However, the tumor suppression was still limited and relative tumor sizes were twice or three times larger. For the combined treatment of PTT/chemotherapy (G5 group), the significant tumor growth inhibition compared with chemotherapy was observed while recurrence of tumor appeared after 8 d. To be noted, when GT was administrated with PTT and chemotherapy (G6 group), the tumors were the smallest and the growth of tumor was inhibited continuously, confirming the best antitumor performance of complementary PTT/chemotherapy.

The immunohistochemical analysis of the dissected tumor tissues was employed to reveal the expression of antioncogene p53. As shown in Figure 6b, widely expressed protein, P53, was visualized in the G2 (GT) and G6 (PTT/chemotherapy) groups while barely any P53 protein-positive area was observed in the tumor tissues of the control group. The therapeutic effect of various treatments was further evaluated by pathological test with hematoxylin-eosin (H&E) staining (Figure 6c). Compared with the control group, more or less apoptosis of tumor cells was observed in all the treatment groups, where shrinking and fragmented cell nuclei were apparent. When all the three treatments (GT/PTT/chemotherapy) were performed, most severe cell apoptosis than any other groups was found. Therefore, the superiority of the triple complementary therapy was further confirmed.

In order to evaluate the in vivo toxicity of starlike Au@SiO2-PGEA2, the body weight of the mice over the duration of the administration was monitored. As shown in Figure S10 (Supporting Information), no obvious loss in body weight was observed. In addition, the earlier reported PGEA-based polyacids do not show histological damage to the major organs such as heart, liver, spleen, lung, and kidney.[27,35] The above results imply that starlike Au@SiO2-PGEA2/p53 complexes possess superior antitumor performance with negligible adverse effects. In addition, the CT and PA images (Figure S11, Supporting Information) resulting from the inherent characteristics of gold nanocaps could be employed to monitor the treatment process real-time to realize imaging-guided therapy.

3. Conclusions

In this work, we successfully synthesized a novel hetero-nanostructure of hollow nanostars with encapsulated gold caps (starlike Au@SiO2). Starlike PbS nanoparticles were adopted as sacrificial templates to shape the starlike hollow capsules and the cross cavity of gold caps. Cationic BUCT-PGEA brushes were utilized to controllably functionalize the starlike Au@SiO2 nanoparticles via surface-initiated ATRP. The resultant starlike Au@SiO2-PGEA exhibited much better gene transfection performance than ordinary spherical analogue. Taking advantage of the photothermal characteristic of unique gold nanocaps and hollow feature of silica nanostars, complementary PTT/chemotherapy could be realized. In addition, PA and CT imaging could realize imaging-guided therapy. The present work offers a versatile model system to fabricate ideal platform for complementary therapy by the rational integration of photothermal gold caps, hollow nanostars, and polycation brushes.

4. Experimental Section

Preparation of Au@SiO2-PGEA Nanohybrids: PbS-Au was prepared and coated with silica to form PbS-Au@SiO2 with different morphologies using PbS nanoparticles as sacrificial templates.[21,36] The PbS templates were then removed completely to result in Au@SiO2 nanoparticles. Thereafter, ATRP initiator was immobilized on the surface of Au@SiO2. Au@SiO2-PGMA nanohybrids were prepared via ATRP of GMA.[28] EA-functionalized Au@SiO2-PGMA (Au@SiO2-PGEA) nanohybrids were finally prepared by the ring-opening reaction of PGMA brushes with EA. The detailed procedures could be found in the Supporting Information.

In Vitro Cytotoxicity, Gene Transfection, and Cellular Uptake: MTT assay was adopted to evaluate the cytotoxicity of Au@SiO2/pDNA in C6 and HEK293 cell lines.[14,15] Transfection assays were performed in both C6 and HEK293 cell lines taking plasmid pRL-CMV as a reporter gene.[24,36] Plasmid pEGFP-N1 was employed to assess gene expression intuitively in C6 cell lines.[24,36] The cellular internalization was evaluated by flow
Photothermal Effect of Starlike Au@SiO$_2$-PGEA2 In Vitro and In Vivo: The photothermal effect of starlike Au@SiO$_2$-PGEA2 was investigated under an 808 nm laser at a power density of 2 W cm$^{-2}$, which is a safe and efficient power density. Temperatures at each time point were recorded by an IR thermal camera. The detailed procedures could be found in the Supporting Information.

Drug Loading and Release: DOX was loaded in the starlike Au@SiO$_2$-PGEA2 to obtain DOX-loaded nanohybrids (DOX@starlike Au@SiO$_2$-PGEA2). Drug release behaviors were investigated in PBS buffer solutions with different pH with or without NIR irradiation. The detailed procedures are described in the Supporting Information.

Combined PTT/GT/Chemotherapy In Vitro and In Vivo: MTT assay was used to evaluate the cell apoptosis induced by p53 gene transfection in the C6 cell line.[27] Starlike Au@SiO$_2$-PGEA2/p53, DOX@starlike Au@SiO$_2$-PGEA2/p53, DOX@starlike Au@SiO$_2$-PGEA2/p53+NIR, and naked p53 were investigated in vitro. For tumor therapy, six groups of tumor-bearing BALB/C nude mice were treated with PBS, starlike Au@SiO$_2$-PGEA2/p53, DOX@starlike Au@SiO$_2$-PGEA2, starlike Au@SiO$_2$-PGEA2+NIR, DOX@starlike Au@SiO$_2$-PGEA2+NIR, and DOX@starlike Au@SiO$_2$-PGEA2/p53+NIR, respectively. H&E and immunohistochemical analysis of the tumors was performed.[39] The detailed procedures are shown in the Supporting Information.

Statistical Analysis: All experiments were repeated at least three times. Data are presented as means ± standard deviation. Statistical significance was set at $p < 0.05$.

Supporting Information
Supporting Information is available from the Wiley Online Library or from the author.
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Conflict of Interest

The authors declare no conflict of interest.

Keywords

drug delivery, gene therapy, hollow nanostars, photothermal gold caps, polycations

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