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Self-destructible polysaccharide nanocomposites with unlockable Au nanorods for high-performance photothermal therapy

Lizhi Song^{1,2,3}, Xin Zhou^{1,2,3}, Xiaoguang Dai^{1,2,3}, Ranran Wang^{1,2,3}, Gang Cheng⁴, Nana Zhao^{1,2,3} and Fu-Jian Xu^{1,2,3}

Abstract

Multifunctional organic/inorganic nanocomposites are highly attractive for effective biomedical applications. In this work, versatile types of self-destructible polysaccharide (dextran or pullulan) nanocomposites (denoted Au@PSa) with adjustable amounts of unlockable Au nanorods (Au NRs) were fabricated as highly efficient photothermal cancer therapy systems. Taking advantage of the acidic endosomes and high concentration of glutathione (GSH) present in cancer cells, the responsive self-destruction of Au@PSa nanocomposites could unlock the abundant encapsulated Au NRs within cells. Notably, the pullulan-based nanocomposites (denoted Au@Pul) demonstrated liver cell-targeting properties, which could enhance the therapeutic effects while minimizing side effects. The strong absorption of the unlocked Au NRs in the near-infrared region was utilized to examine the photothermal performance. The Au@PSa nanocomposites with a moderate amount of Au NRs in the matrix exhibited very impressive photothermal effects in tumor therapy, where the encapsulated Au NRs were unlocked in the tumor region to realize the observed high performance. The proposed system could be further extended, as the polysaccharides were functionalized with amino groups. The current work provides a facile strategy to construct flexible therapeutic platforms with responsive self-destruction features.

Introduction

Cancer is still a deadly disease worldwide and is the leading cause of death¹. A variety of therapeutic modalities, such as chemotherapy and photothermal therapy (PTT), have been developed to conquer the complexity of tumor and cancer metastasis^{2–4}. As one of the most appealing strategies, PTT through the conversion of near-infrared (NIR) light into heat possesses the advantage of negligible damage to normal cells^{5–7}. NIR light-absorbing

plasmonic nanoparticles have been successfully employed for effective PTT, and most of these nanoparticles exploit passive targeting by the enhanced permeation and retention (EPR) effect^{8–11}. Surface ligands for active cell targeting could further facilitate the preferential accumulation of nanomaterials^{12–14}. In addition to targeting, stimuli-responsive nanomaterials that can distinguish between aberrant tumor regions and normal tissues to minimize side effects have been designed^{15–20}. In addition, taking advantage of the properties of tumor intracellular environments, such as the presence of acidic endosomes (5.0–5.5) and a high concentration of glutathione (GSH, 2–10 mM), stimuli-responsive destructible nanomaterials are also ideal for achieving enhanced performance^{21,22}.

Correspondence: Nana Zhao (xufj@mail.buct.edu.cn) or F.-J. Xu (zhaonn@mail.buct.edu.cn)

¹State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, 100029, China

²Key Laboratory of Carbon Fiber and Functional Polymers, Beijing University of Chemical Technology, Ministry of Education, Beijing, 100029, China

Full list of author information is available at the end of the article.

These authors contributed equally: L. Song, X. Zhou

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Natural polysaccharides (PSa), including chitosan, dextran (Dex), and pullulan, are renewable materials with low toxicity, degradability, bioactivity, and excellent biocompatibility^{23,24}. Due to the existence of abundant hydroxyl and other functional groups, PSa could be used to construct intriguing therapeutic platforms after appropriate modification^{24,25}. Particularly, pullulan possesses an inherent liver-targeting property, and pullulan-based conjugates have been proven superior in cell-targeting biomedical applications^{26–28}. Possessing tunable longitudinal surface plasmon resonance (LSPR), gold nanorods (Au NRs) demonstrate strong absorption in the desirable NIR region^{29,30}. Versatile therapy platforms based on Au NRs have been extensively studied and demonstrate great potential in cancer theranostics^{31–33}. It was reported that chitosan/Au NR nanospheres possess advantages of both chitosan and Au NRs in imaging-guided cancer therapy^{34,35}. However, only one Au NR was loaded in each nanosphere, and this method was limited to chitosan. If more Au NRs could be loaded in one nanocapsule without severe aggregation to maintain a distinct LSPR peak in the NIR region, the relatively higher concentration of Au NRs would produce a better therapeutic performance. To explore diverse PSa types and enhance the photothermal performance, it is desirable to develop a general strategy to synthesize different kinds of PSa nanocomposites with a suitable loading amount of NRs.

Herein, we propose a flexible strategy to construct versatile types of self-destructible PSa nanocomposites (Au@PSa) with adjustable amounts of unlockable Au NRs for efficient photothermal cancer therapy (Fig. 1). Water-soluble Dex and liver-targeting pullulan were explored as model systems. A variety of Au@PSa nanocomposites were readily achieved by the addition of Au NRs and the Schiff base crosslinking of aminated PSa. The acid-labile Schiff base bonds impart the resulting Au@PSa nanocomposites with the ability to self-destruct within cancer cells. In addition, the interaction between Au NRs and GSH could further accelerate the breakdown of the Au@PSa nanocomposites to unlock the abundant Au NRs³⁶. Taking the enhanced photothermal effects from the large amount of unlocked Au NRs into account, the PTT performance of the Au@PSa nanocomposites is worth examination. Compared with the previous work in which more than one Au NR was encapsulated in mesoporous silica nanobeads³⁷, diverse natural PSa provide not only a responsive self-destructible matrix but also inherent targeting properties, which could enhance the therapeutic effects while minimizing side effects. The responsive self-destruction and high-performance PTT of the Au@PSa nanocomposites were investigated in detail.

Materials and methods

Materials

Chloroauric acid, ascorbic acid, and glutaraldehyde (GA, 25 wt%) were obtained from Sinopharm Group Co. Ltd (China). Silver nitrate (99.8%), sodium borohydride (98%), cetyltrimethylammonium bromide (CTAB, 99%), 1,1'-carbonyldiimidazole (CDI, 97%), Dex (Mw ~23,800 g/mol), anhydrous dimethyl sulfoxide (DMSO), and ethylenediamine (ED, >98%) were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Pullulan (Mw ~12,000 g/mol) was obtained from International Laboratory (USA). Ethylenediaminetetraacetic acid (EDTA) disodium salt was obtained from Beijing Chemical Works (Beijing, China). Fluorescein isothiocyanate isomer I (FITC, 95%) was obtained from Energy Chemical Co., Ltd (Shanghai, China). Fluorescein diacetate (FDA), propidium iodinate, (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), penicillin, and streptomycin were purchased from Sigma-Aldrich Chemical Co. HepG2 and C6 cell lines were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA).

Preparation of PSa-NH₂

For the preparation of Dex-NH₂, 0.5 g of Dex was dissolved in 3.5 mL of anhydrous DMSO, and 0.5 g of CDI in 1.5 mL of anhydrous DMSO was added dropwise. The resultant mixture was degassed by bubbling nitrogen through it for 10 min and kept at room temperature for 4 h. Then, 1.48 mL of ethylenediamine (ED) was added dropwise into the above solution under vigorous stirring. The reaction mixture was degassed by bubbling nitrogen through it for 10 min and stirred at room temperature for 48 h. After dialysis (MWCO 3500) against deionized water at room temperature for 48 h, the final product, Dex-NH₂, was freeze dried. For the preparation of Pul-NH₂, 1 g of pullulan was dissolved in 20 mL of anhydrous DMSO. Then, 1 g of CDI in 6 mL of anhydrous DMSO was added dropwise. The resultant mixture was kept at 37 °C for 6 h. Thereafter, 8.25 mL of ED was added, and the solution was stirred at 37 °C for 24 h. The final product, Pul-NH₂, was dialyzed and freeze dried.

Preparation of Au@PSa nanocomposites

Au@PSa nanocomposites were prepared using the nonsolvent-aided counterion complexation method. Au NRs were synthesized using the typical seed-mediated growth method reported earlier³³. For the preparation of Au@Dex nanocomposites, 1 mL of Dex-NH₂ aqueous solution (0.3 mg/mL) was mixed with 1 mL of Au NR aqueous solution (0.059 mg/mL for Au@Dex-1, 0.12 mg/mL for Au@Dex-2 and 0.24 mg/mL for Au@Dex-3) and 1 mL of EDTA aqueous solution (1.2 mg/mL for Au@Dex-1

Fig. 1 Schematic illustration of the preparation processes of the self-destructible Au@PSa nanocomposites as high-performance therapeutic platforms

and Au@Dex-2 and 1.6 mg/mL for Au@Dex-3). The mixture was stirred vigorously at room temperature for 30 min. Then, 1.5 mL of ethanol was added dropwise under vigorous stirring, and the solution turned opalescent. Finally, 50 μ L of GA solution (25 wt%) was added at room temperature. After 4 h, the resultant Au@Dex nanocomposites were collected by centrifugation and washed with deionized water four times to remove CTAB and any residual reactants.

For the preparation of Au@Pul nanocomposites, 2 mL of Pul-NH₂ solution (0.5 mg/mL) was mixed with 2 mL of Au NR solution (0.059 mg/mL for Au@Pul-1, 0.12 mg/mL for Au@Pul-2, and 0.24 mg/mL for Au@Pul-3) and 2 mL of EDTA solution (1.5 mg/mL for Au@Pul-1 and 1.0 mg/

mL for Au@Pul-2 and Au@Pul-3). The final mixture was stirred vigorously at room temperature for 30 min. Then, 3 mL of ethanol was added dropwise under vigorous stirring. Finally, 100 μ L of GA solution (25 wt%) was added. After 4 h, the Au@Pul nanocomposites were collected by centrifugation.

Responsive self-destruction of the Au@PSa nanocomposites

The destruction of nanocomposites was carried out in a pH 5.0 solution. First, 25 μ L of a Au@PSa suspension (2 mg/mL) was added into 975 μ L of a pH 5.0 solution in the presence or absence of 10 mM GSH and then incubated at 37 $^{\circ}$ C for 24 or 48 h.

Cellular internalization

Cellular internalization was evaluated in HepG2 and C6 cell lines by flow cytometry, fluorescence microscopy, and confocal laser scanning microscopy (CLSM). FITC was used to conjugate the Au@PSa nanocomposite for visualization. The synthetic protocol was based on a method published elsewhere³⁸. First, 40 μL of FITC in DMSO solution (1 mg/mL) was added to 20 mL of Au@Dex-2 or Au@Pul-2 solution (0.05 mg/mL, pH 11), and the solution was then stirred for 1 h at 40 °C. The FITC-labeled Au@Dex-2 and Au@Pul-2 nanocomposites were collected by centrifugation, washed with deionized water five times and dried under vacuum. The detailed procedures are shown in the Supporting Information.

Bio-TEM observation of Au@PSa-treated HepG2 cells

Bio-transmission electron microscope (Bio-TEM) was carried out to observe the distribution of Au@PSa in treated HepG2 cells following the previously reported procedure³⁹. The details are shown in the Supporting Information.

In vitro cytotoxicity assay

The cytotoxicity of Au@PSa was evaluated by the MTT viability assay in HepG2 cell lines⁴⁰. The detailed procedures are shown in the Supporting Information.

Photothermal effect of Au@PSa

Three milliliters of Au@PSa solution (200 $\mu\text{g/mL}$) in a quartz cuvette was irradiated by an 808 nm laser (2 W/cm, 5 min), and the temperature was recorded by an IR thermal camera. HepG2 cells were cultured with Au@PSa at various mass concentrations for in vitro photothermal experiments. For in vivo NIR thermal imaging, tumor-bearing nude mice were injected with 150 μL of Au@Pul-2 solution (3 mg/mL). The detailed procedures are shown in the Supporting Information.

In vivo PTT

HepG2 tumor-bearing nude mice were treated by injection of 150 μL of phosphate-buffered saline (PBS) (control group), Au@Pul-2 (3 mg/mL), and Au@Pul-2 (3 mg/mL) with irradiation by an NIR laser (PTT group, 2 W/cm, 10 min). The control group and PTT group were irradiated by an 808 nm laser (2 W/cm², 10 min) only once after injection. Hematoxylin and eosin (H&E) staining was carried out according to the standard protocols described in the previous work⁴¹. The detailed procedures are shown in the Supporting Information.

Statistical analysis

All experiments were repeated at least three times. Data are presented as the mean $\pm$ standard deviation. Statistical significance ($p < 0.05$) was evaluated by a *t* test when two

groups of samples were compared, and statistical significance was set at $p < 0.05$.

Results and discussion

Synthesis and characterization of the Au@PSa nanocomposites

For the synthesis of the Au@PSa nanocomposites, the hydroxyl groups of PSa (Dex or pullulan) were first activated by CDI and reacted with ED to produce PSa-NH₂ with primary amino groups (Fig. 1)^{26,42}. Typical proton nuclear magnetic resonance (¹H NMR) spectra of Dex-NH₂ and Pul-NH₂ are shown in Figure S1 in the Supporting Information. Based on the ¹H NMR spectra, amino groups were successfully conjugated to the glucose units of Dex and pullulan. Then, Au@PSa nanocomposites were synthesized by employing PSa-NH₂, Au NRs with an LSPR peak at 780 nm and EDTA as the starting materials. EDTA attached to the Au NR surfaces through electrostatic interactions with the stabilizer CTAB. With the addition of ethanol, which is a nonsolvent for both PSa and EDTA, counterion condensation of cationic PSa-NH₂ and anionic EDTA occurred around the Au NRs as the nucleation center^{35,37}. After the formation of Schiff base bonds between the amino groups of PSa-NH₂ and the aldehyde groups of GA, EDTA was removed through centrifugation, and Au NRs were encapsulated in the PSa-NH₂ matrix to form Au@PSa nanocomposites.

As displayed in Fig. 2, versatile Au@PSa nanocomposites with adjustable amounts of Au NRs were readily achieved by modulating the concentrations of Au NRs, PSa-NH₂, and EDTA. Both Au@Pul and Au@Dex nanocomposites with different amounts of encapsulated Au NRs, denoted Au@Dex-1, Au@Dex-2, and Au@Dex-3 (Fig. 2a) and Au@Pul-1, Au@Pul-2, and Au@Pul-3 (Fig. 2b), were fabricated successfully. The sizes of the Au@Dex nanocomposites were comparable (89 ± 6 , 96 ± 4 , and 91 ± 15 nm, respectively), while the Au@Pul counterparts were slightly larger (117 ± 22 , 113 ± 18 , and 118 ± 21 nm, respectively). The surface potentials of all the Au@PSa nanocomposites were approximately 30 mV (Figure S2, Supporting Information). As displayed in Fig. 2c, the LSPR absorption peaks of Au@Dex-1, Au@Dex-2, and Au@Dex-3 were 780, 767, and 707 nm, respectively. In other words, with an increasing amount of encapsulated Au NRs, an obvious blueshift of the LSPR peak occurred. Notably, the LSPR peak of Au@Dex-3 was almost distorted due to its largest degree of encapsulated Au NR aggregation, which was observed and confirmed by a discrete dipole approximation calculation in the previous report⁴³. The same tendency was observed in the case of the Au@Pul nanocomposites from Au@Pul-1 to Au@Pul-3, where the LSPR absorption peak moved from 760 to 660 nm (Fig. 2e). The encapsulation of more Au NRs in the nanocomposites caused the absorption

Fig. 2 Characterization of Au@PSa nanocomposites. **a, b** TEM images of different Au@PSa nanocomposites; **c, e** UV-Vis spectra of the Au NRs and Au@PSa nanocomposites; and **d, f** temperature variations of water and Au@PSa solutions (200 $\mu\text{g/mL}$) upon irradiation at 808 nm (2 W/cm²)

spectrum to approach that of spherical Au nanoparticles, and a blueshift occurred. The photothermal properties of the Au@PSa nanocomposites were further investigated, where Au@PSa suspensions with a mass concentration of 200 $\mu\text{g/mL}$ were irradiated by an NIR laser at 808 nm (Fig. 2d, f). At the same mass concentration, Au@Dex-2

and Au@Pul-2 demonstrated higher temperatures due to their suitable absorption intensities and loading amounts of Au NRs. Thus, the Au@Dex-2 and Au@Pul-2 nanocomposites with moderate amounts of Au NRs were selected as the model system for the following studies. The weight ratio of Au NRs encapsulated in Au@Pul-2

Fig. 3 Responsive destruction of Au@Pul-2 nanocomposites. (a) TEM images of Au@Pul-2 incubated at pH = 5.0 in the absence and presence of 10 mM GSH. (b) Temperature variation of Au@Pul-2 solutions (200 $\mu\text{g/mL}$) before and after incubation at pH = 5.0 for 4 h in the absence and presence of 10 mM GSH upon irradiation at 808 nm (2 W/cm²)

and Au@Dex-2 was comparable (~40%) based on thermogravimetric analysis (Figure S3, Supporting Information).

Responsive destruction of the Au@PSa nanocomposites

As a responsive therapeutic platform, the self-destruction of the nanocomposites in an aberrant tumor intracellular environment would help to enhance the photothermal effect⁴⁴. Fig. 3a illustrates the morphological evolution of Au@Pul-2 when incubated at pH 5.0 to simulate the acidic endosomes and lysosomes (5.0–5.5). The high concentration of GSH present within cancer cells was also considered to unlock the Au NRs by accelerating the destruction of the Au@PSa nanocomposites. After incubation at pH 5.0 for 1 day, the matrix of the Au@Pul-2 nanocomposite seemed to dissolve and be destroyed, probably due to the breakage of

the Schiff base bonds. After 2 days, more of the matrix dissolved, while looser complexes of pullulan-Au were observed. When GSH was added to the acidic solution, the Au@Pul-2 nanocomposite further broke down into discrete Au NRs. The Au@Dex-2 nanocomposite underwent similar decomposition processes, and more readily unlocked Au NRs appeared in the presence of GSH (Figure S4, Supporting Information). The strong interaction between the Au NRs and thiol groups of GSH contributed to the unlocking of Au NRs from the matrix^{45,46}, which in turn accelerated the responsive destruction of the Au@PSa nanocomposites. The discrete Au NRs unlocked from the nanocomposites were anticipated to enhance the photothermal effect because of their favorable optical properties. Figure 3b displays the temperature elevation of the Au@Pul-2 suspension before and after self-destruction. It is evident that the temperature

Fig. 4 Cellular internalization of FITC-labeled Au@PSa nanocomposites. a Fluorescence images and flow cytometry analysis results of C6 and HepG2 cells treated with Au@Dex-2 and Au@Pul-2 for 4 h. **b** CLSM images of HepG2 cells treated with Au@Dex-2 and Au@Pul-2 for 0.5 and 4 h, where FITC-labeled nanocomposites are shown in green and DAPI-labeled nuclei are shown in blue

elevation of the self-destructed sample at pH 5.0 (42 °C) after irradiation is higher than the original one (39 °C). In the presence of GSH, the temperature elevation further increased to 46 °C, confirming the enhanced photothermal effect of the unlocked Au NRs from the Au@Pul-2 nanocomposite. Therefore, GSH could pull the encapsulated Au NRs out of the nanocomposites through the formation of Au-S bonds so that the LSPR peaks of the self-destructed sample redshifted to result in an enhanced photothermal effect.

Cellular internalization of the Au@PSa nanocomposites

Pullulan is considered to possess a specific affinity for hepatoma cells with asialoglycoprotein receptor (ASGPR) overexpression⁴⁷. In this study, the anticipated hepatoma-targeting property of the Au@Pul nanocomposite was verified by comparison of cellular internalization of Au@Dex-2 and Au@Pul-2 in hepatoma HepG2 cells. Glioma C6 cells without ASGPR were employed as the control. As shown in Fig. 4a, both FITC-labeled Au@Dex-2 and Au@Pul-2 were internalized by C6 and HepG2 cells after 4 h of incubation. It was observed that the green fluorescence signal from the Au@Pul-2-treated HepG2 cells was significantly stronger than that of the other groups. To quantitatively compare the cellular uptake, flow cytometry was performed to analyze the internalization ratio. In the C6 cell line, Au@Dex-2 and Au@Pul-2 demonstrated respective internalization ratios of 21% and 26%, which are relatively low and comparable to one another. In contrast, Au@Pul-2 displayed a much higher internalization ratio (90%) than Au@Dex-2 (24%) in the HepG2 cell line, proving that the Au@Pul-2 nanocomposite preserves the excellent hepatoma-targeting ability of pullulan. Therefore, the Au@Pul-2 nanocomposite was assumed to facilitate targeted cancer therapy.

The internalization of Au@Dex-2 and Au@Pul-2 in HepG2 cells was also monitored with real-time CLSM (Fig. 4b). The nucleus was stained with DAPI (4',6-diamidino-2-phenylindole, blue fluorescence), while FITC-labeled Au@Dex-2 and Au@Pul-2 were visualized in green. The signals of the Au@Dex-2 nanocomposite were weak after 0.5–4 h of incubation, while the Au@Pul-2 nanocomposite was readily internalized in HepG2 cells. An obvious time-dependent trend was observed. After 4 h of incubation, a large portion of the Au@Pul-2 nanocomposite accumulated around the nuclei, exhibiting strong fluorescence signals, while little fluorescence was observed for Au@Dex-2, indicating that Au@Pul-2 entered the cells much faster. Considering the fact that the particle sizes and surface potentials of Au@Dex-2 and Au@Pul-2 are similar (Fig. 2a, b and Figure S2, Supporting Information), the dramatic difference in cellular uptake was not caused by the surface morphology or size.

Fig. 6 In vitro photothermal effect of Au@PSa nanocomposites. **a** Particle sizes of Au@Dex-2 and Au@Pul-2 in medium supplemented with 10% fetal bovine serum (FBS) (mean $\pm$ SD, $n = 3$). **b** Cell viability assay of HepG2 cells treated with Au@Dex-2 and Au@Pul-2 at various concentrations without and with NIR irradiation (mean $\pm$ SD, $n = 6$; *significant difference, $P < 0.05$). **c** Representative fluorescence images of FDA-PI-stained HepG2 cells under irradiation and treatment with Au@Pul-2 (30 μ g/mL) in the absence and presence of irradiation at 808 nm

These findings further confirmed the specific affinity of Au@Pul-2 for hepatoma cells. In addition, the internalization of Au@Pul-2 was believed to undergo receptor-mediated endocytosis through binding with over-expressed ASGPR⁴⁸.

The internalized amount of Au@Dex-2 and Au@Pul-2 in HepG2 cells was quantitatively analyzed by inductively coupled plasma mass spectrometry (ICP-MS). As shown in Fig. 5a, a significant difference was found between the Au contents of the HepG2 cells treated with Au@Dex-2 and Au@Pul-2, also suggesting that Au@Pul-2 could efficiently enter HepG2 cells. Furthermore, the Au@Dex-2-treated and Au@Pul-2-treated HepG2 cells were sectioned to visualize the distribution of the Au@PSa nanocomposites. Judging from the Bio-TEM images of sectioned HepG2 cells, both Au@PSa nanocomposites were mainly localized in the cytoplasm (Fig. 5b, c), and larger amounts of Au@Pul-2 over Au@Dex-2 were found in the cell, in agreement with the ICP-MS results (Fig. 5a).

In vitro photothermal effect of the Au@PSa nanocomposites

The strong LSPR absorption and self-destruction features of Au@Pul-2 and Au@Dex-2 motivated us to exploit their photothermal effects. The weight ratio of encapsulated Au NRs was comparable between Au@Pul-2 and Au@Dex-2 based on the results of thermogravimetric analysis (Figure S4, Supporting Information). Dynamic

light scattering measurements revealed that the hydro-dynamic sizes of Au@Dex-2 and Au@Pul-2 in the culture medium supplemented with 10% fetal bovine serum were comparable (approximately 175 nm) and quite stable during incubation (Fig. 6a). Moreover, the surface charges of Au@Dex-2 and Au@Pul-2 were also equivalent (Figure S2, Supporting Information). Therefore, it is reasonable to compare the photothermal properties of these two kinds of Au@PSa nanocomposites. As demonstrated in Fig. 2d, f, the temperature elevation of the Au@Dex-2 and Au@Pul-2 suspensions at the same concentration of 200 μ g/mL were also comparable (approximately 38 °C and 39 °C, respectively), suggesting that the nanocomposites have similar photothermal effects in solution.

To investigate the in vitro photothermal properties, an MTT assay was carried out to evaluate the cell viability under treatment by Au@Dex-2 and Au@Pul-2 in the HepG2 cell line in the absence or presence of NIR irradiation. As shown in Fig. 6b, the cell viabilities mediated by Au@Dex-2 and Au@Pul-2 were observed to change slightly with increasing concentration without NIR irradiation. At the high concentration of 60 μ g/mL, the viability of the HepG2 cells was still above 80%, consistent with the good biocompatibilities of Dex and pullulan. Given the impressive photothermal performances (Fig. 2d, f), Au@Dex-2 and Au@Pul-2 are considered promising candidates for efficiently killing cancer cells under NIR irradiation. A preliminary in vitro experiment of the

Fig. 7 In vivo PTT. **a** Infrared thermal images and **(b)** temperature variation in HepG2 tumor-bearing mice after injection with PBS (control) or Au@Pul-2 in the presence of 808 nm irradiation (2 W/cm^2). **c** Time-dependent growth curves, weights and photographs of tumors after the different treatments. H&E staining images of the tumors **(d)** and **(e)** major organs (heart, liver, spleen, kidney, and lung) of mice. Scale bars: $50 \mu\text{m}$

viability of HepG2 cells under NIR irradiation was quantitatively conducted. As shown in Fig. 6b, the cell viabilities under NIR irradiation decreased, unlike the ones without irradiation. Compared with the Au@Dex-2-treated cells, the cells treated with Au@Pul-2 exhibited a substantially lower viability at the same concentration. In particular, significant differences between the two groups were observed when the concentration was 30 or 60 $\mu\text{g}/\text{mL}$. The cell viability of HepG2 cells was as low as 19% when the concentration of Au@Pul-2 was 60 $\mu\text{g}/\text{mL}$. These results confirmed the superior photothermal effect of Au@Pul-2, which might be caused more by its effective internalization and the specific affinity of Au@Pul-2 for HepG2 cells. After the HepG2 cells treated with the Au@Pul-2 nanocomposite were exposed to the NIR laser, considerable cell death was observed in the live/dead staining images with FDA and PI, where most HepG2 cells remained viable under laser irradiation only or when treated by Au@Pul-2 without irradiation (Fig. 6c). These intuitive observations further suggest the excellent targeting ability and photothermal characteristics of the Au@Pul-2 nanocomposite for killing HepG2 cells.

In vivo PTT

To validate the feasibility of Au@Pul-2 for application in in vivo PTT, tumor-bearing nude mice were injected with Au@Pul-2 or PBS (control), and then the tumors were irradiated by an 808 nm laser ($2\text{ W}/\text{cm}^2$). The real-time IR thermal images with intuitive temperature changes reveal the photothermal effect of Au@Pul-2 (Fig. 7a). As displayed in Fig. 7b, the corresponding temperature elevation in the Au@Pul-2 group increased to approximately 24°C , which could be utilized to suppress tumor growth. The control group showed negligible changes, in accordance with the photographs and temperature variation of the tumor region.

Encouraged by the above effective photothermal performances, we verified the hypothesis that Au@Pul-2 could be employed for in vivo PTT with satisfactory anticancer effectiveness. Liver tumor-bearing nude mice were randomly divided into three groups for different administrations: control (PBS only), Au@Pul-2, and Au@Pul-2 + NIR (PTT group). The volumes of the tumors were recorded every 2 days to monitor the growth rate. After 10 days, all the mice were euthanized, and the tumors of the different groups were weighed and imaged (Fig. 7c). The tumors of the control group were found to grow very fast, manifesting the typical high-proliferation-rate behavior of liver cancer. Tumor growth in the Au@Pul-2 group was not much different than that in the control group, consistent with the low cytotoxicity of Au@Pul-2 (Fig. 6b). However, for the PTT group, apparent tumor growth inhibition was observed, in accordance with the excellent photothermal performance

of Au@Pul-2 (Fig. 6). Judging from the cellular internalization and in vitro photothermal results (Figs. 4–6), the Au@Pul-2 nanocomposite possesses an excellent targeting capability for hepatoma cells, which might facilitate the accumulation and retention in the region of hepatoma to enhance the PTT effectiveness. Remarkably, the tumors of two mice in the PTT group finally disappeared after the treatment, suggesting the superiority of PTT with the self-destructible Au@Pul-2 nanocomposite. The pathological test revealed by H&E staining of tumor slices further confirmed the therapeutic effect (Fig. 7d). Massive shrinking and fragmented cell nuclei were observed in the PTT group, while negligible tumor cell apoptosis was found in the control and Au@Pul-2 groups, providing evidence for safe PTT by the Au@Pul-2 nanoparticles with a moderate dose of irradiation.

Moreover, histological analysis of the major organs, including the heart, liver, spleen, kidney, and lung, was also conducted to evaluate the side effects of PTT. As displayed in Fig. 7e, the images of the H&E-stained major organs in the PTT group did not present any abnormalities compared with those of the control group. The body weight of all the three groups of mice increased during the administration period and displayed no distinct differences (Figure S5, Supporting Information), validating the compatibility and feasibility of Au@Pul-2 for PTT. Therefore, Au@Pul-2-mediated PTT would be impactful in the treatment of high-proliferation-rate HepG2 tumors.

Precise and accurate imaging during the therapeutic processes could provide valuable feedback for obtaining the best antitumor effect⁴⁹. Taking advantage of their NIR absorption, Au NRs could realize PA imaging, an emerging imaging modality that offers sensitive signals of tumors located within a few centimeters⁵⁰. The feasibility of Au@Pul-2 for PA imaging was also testified in vitro and in vivo. As the concentration of Au@Pul-2 increased, the corresponding intensity of the PA signals increased, and stronger PA images were accordingly observed (Figure S6a). After Au@Pul-2 suspensions were injected into HepG2 tumor-bearing nude mice, remarkably enhanced PA signals compared with those of the original tumor region were obtained (Figure S6b), confirming the tremendous potential of Au@Pul-2 for PA imaging-guided PTT.

Conclusions

In summary, versatile types of Au@PSa nanocomposites (Au@Pul and Au@Dex) consisting of adjustable amounts of Au NRs and pullulan or Dex were prepared successfully through a facile strategy. In this regard, the system could be further extended, as the PSa were functionalized with amino groups. Au@PSa nanocomposites possess a responsive self-destruction characteristic that enables unlocking of the Au NRs from the matrix to achieve

better photoperformance. Because of the good biocompatibility and liver-targeting properties of pullulan, the Au@Pul nanocomposites exhibited an excellent affinity for HepG2 cells and superior in vivo photothermal performance. It is worth mentioning that the abundant hydroxyl groups of the PSa could be utilized to introduce more functionality, while the versatile Au@PSa matrix could be tailored to other biomedical applications. Therefore, the present study not only offers a general approach for the synthesis of Au@PSa nanocomposites but also opens a new avenue for the rational design of multifunctional therapeutic platforms.

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Author details

¹State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, 100029, China. ²Key Laboratory of Carbon Fiber and Functional Polymers, Beijing University of Chemical Technology, Ministry of Education, Beijing, 100029, China. ³Beijing Laboratory of Biomedical Materials, Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, Beijing, 100029, China. ⁴Department of Chemical Engineering, University of Illinois at Chicago, Chicago, IL 60607, USA

Conflict of interest

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