BSA-Coated Gold Nanorods for NIR-II Photothermal Therapy

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Abstract

The second near infrared window is considered to be the optimal optical window for medical imaging and therapy as the deep tissue penetration. A series gold nanorods with large aspect ratio have been synthesized. Strong plasma absorption in the second near infrared window from 1000 nm to 1300 nm could be observed. The biocompatibility of the synthesized gold nanorods is dramatically improved via coating by bovine serum albumin (BSA), while the optical properties of which remains. The breast cancer tumor-bearing mouse could be well treated by the prepared gold nanorods with the NIR-II light intensity as low as 0.75 W/cm^2 . In summary, these results prove the feasibility of using low dose to treat tumor in the NIR-II region.

Introduction

Gold nanoparticles are widely used in cancer therapy due to as the surface plasmon resonance (SPR) effect, gold nanoparticles can efficiently absorb photon energy and convert which to heat [1, 2]. Therefore, various gold nanoparticles with adjustable size and morphology are widely used for thermal ablation of tumors, for instance gold nanorods, gold nanoshells, and gold nanocages [3–5]. In particular, gold nanorods with adjustable anisotropic shape and size have been widely studied since their excellent photothermal stability, biocompatibility, and strong absorption in NIR region [6]. It is well known that near-infrared light could penetrate biological tissues more effectively than visible light, as the longer the light wavelength, the lower the scattering loss [7]. In addition, the rod-shaped nanoparticles have been found with dramatically enhanced tumor permeability and longer blood circulation time, resulting higher tumor accumulation [8, 9]. However, the significance drawback for applying gold nanorods to photothermal therapy (PTT) is the high-power laser irradiation, which would induce highly damage of normal tissue [10]. It has been proven that the PTT in the second near infrared window (NIR-II, 1000 nm- 1700 nm) has much greater tissue penetration depth than that in NIR-I (700 nm-1000 nm), as the highest maximum allowable exposure (MPE) intensity and the low light scattering in NIR-II [11–14]. Therefore, the PTT nano-platform in the NIR-II is expected to achieve more effective PTT treatment of tumor and to have great clinical application potential for more complex tumor therapy. Here, we report the synthesis of gold nanorods by the seedless method with absorption peaks in the second window of near-infrared (1000 nm – 1300 nm). Surface modification was introduced via coating with BSA to reduce the cytotoxicity. The aspect ratio of prepared gold nanorods (AuNR@BSA) were chracterized by TEM. The breast cancer tumor-bearing mouse model was used to test the photothermal therapeutic effect of AuNR@BSA. We found that the tumor could be well treated with light intensity as low as 0.75 w/cm^2 .

Materials And Methods

Materials

Gold chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), Hexadecyl trimethyl ammonium bromide (CTAB), Nitric acid (GR,65-68%) and Hydrogen peroxide solution (GR,30%) were received from Shanghai Aladdin biological

technology Co. Ltd. Sodium borohydride (NaBH_4) and silver nitrate (AgNO_3) were received from Shanghai Lingfeng Chemical Reagent Co. Ltd. Hydrochloric acid (HCl) were received from Dongguan Dongjiang Chemical Reagent Co. Ltd. Hydroquinone were received from Energy Chemical. Bovine Serum Albumin were obtained from Sigma-Aldrich. Sodium hydroxide were received from Greagent.

RPMI 1640 Medium and Penicillin-Streptomycin were purchased from HyClone. Phosphate buffered solution (PBS) were purchased from Corning. Pancreatin were purchased from Coolaber. Foetal bovine serum (FBS) were purchased from Gibco. 4T1 cells were provided by Research Center for Biomedical Optics and Molecular Imaging in the Shenzhen Institute of Advanced Technology, Chinese Academy of Science. Dojindo Chemical Technology (Shanghai) Co., Ltd supplied Cell Counting Kit-8 for Cell proliferation and toxicity test. The Millipore ultrapure water was used throughout the experiment.

Preparation of AuNR@CTAB nanoparticles

The synthesis of gold nanorods is conducted as follows: 0.4 mL of $\text{HAuCl}_4(\text{aq})$ (10 mM) and 10 mL of $\text{CTAB}(\text{aq})$ (0.1 M) were added to 23-33 μL of $\text{AgNO}_3(\text{aq})$ (100 mM). Then, 10-30 μL of HCl (1.2 M) and 525 μL of aqueous hydroquinone (0.1 M) were added to the growth solution under gentle mixing. The color of the growth solution turned from orange to a very light yellow color. After 15 min of stirring, 10-40 μL of freshly prepared ice-cold $\text{NaBH}_4(\text{aq})$ (10 mM) solution was injected into the growth solution. The mixture was stirred for 30 s and aged for 18 h at room temperature. The AuNR@CTAB was then washed by PBS twice.

Preparation of AuNR@BSA

First, we add a certain amount of CTAB to adjust its concentration in AuNR@CTAB solution to 1 mM, and then ultrasound until CTAB is completely dissolved. 3 mL AuNR@CTAB is slowly added to 3 mL BSA solution bovine serum albumin (10 mg/mL), and the mixed solution is sonicated for 30 min. After centrifugation at $9,500 \times r$ for 40 min, the supernatant was replaced with 6 mL BSA solution (5 mg/mL), and then the pH value was adjusted to 11-12 with sodium hydroxide (2 M), stirred for at least 18 h. After that, the synthesized AuNR@CTAB was centrifuged at $9,500 \times r$ for 40 min, then washed twice with PBS, and dissolved in PBS for further use.

Characterizations of AuNR@CTAB and AuNR@BSA Nanoparticles

The morphology analysis of gold nanorods was obtained by Beijing Zhongke Baice Co., Ltd. through Talos F200X electron microscope to obtain transmission electron microscope (TEM) images. Zetasizer Nano ZS (Malvern, UK) was used to study the size distribution and zeta potential of various nanoparticles by dynamic light scattering (DLS). The UV-Vis absorption spectrum was determined by UV-2700 Ultraviolet-Visible Spectrophotometer (SHIMADZU, Japan).

For the morphology characterization of AuNR@BSA inside tumor, 100 μL of AuNR@BSA (OD = 25) were injected into tumor sites with irradiation about 10 min, after that the treated tumors were collected. Non-

treated tumor was collected as control. The collected tumors were incubated in 2.5% glutaraldehyde solution (Coolaber.co., Beijing, China) for transmission electron microscopy (Beijing Zhongke Baice Co., Ltd).

Measurement of the photothermal performance of AuNR@CTAB and AuNR@BSA

The gold nanorod solution was diluted to different OD (0.5, 1, 1.5, and 2), and the PBS was used as a blank control. The gold nanorods (500 μL) were irradiated with a 1064 nm laser (Haoliangtech, Shanghai, China) at a power intensity of 0.35 to 1 W/cm^2 for 30 min. The temperature was recorded with an infrared thermal imager (FLUKE TI25).

Photostability of AuNR@BSA

To test the photostability, the absorption spectra of AuNR@BSA were measured as a function of the irradiation time. The AuNR@BSA (OD=1) were irradiated under NIR laser (1064 nm, 0.5 W/cm^2). From 0 to 10 min, the spectrum was recorded every minute. The photothermal cycle test was also carried out as the AuNR@BSA solution (0.5 mL) irradiated with every 10 minutes laser irradiations on and off (1064 nm, 0.5 W/cm^2), and the temperature change was recorded.

Cellular Culture

Murine breast cancer cell line (4T1 cells) was cultured in RPMI 1640 containing 10% FBS and 100 U/mL penicillin or 100 $\mu\text{g}/\text{mL}$ streptomycin. The culture environment is 37 °C, and the humidification condition is 5% CO_2 .

In vitro cytotoxicity assessment of gold nanoparticles

The CCK-8 assay was used to identify the cytotoxicity of gold nanorods. 4T1 cells were pre-seeded into 96-well plates (5×10^3 per well) and incubated for 24 h. Subsequently, 10 μL of various concentrations of AuNR@CTAB and AuNR@BSA were added and incubated for another 24 h. After washing with PBS twice, 10 μL CCK-8 solution was added to each well and incubated for 40 min, followed by measuring the absorbance at 450 nm with a microplate reader.

For phototoxicity, 4T1 cells were pre-seeded into a 96-well plate (5×10^3 per well) and incubated for 24 h, then the cells were irradiated with NIR laser (1064 nm, 0.75 W/cm^2 , 10 min) and further incubated for 24 h. After that, 10 μL CCK-8 solution was added into each well and incubated for a further 40 min at 37 °C. Then, a microplate reader was used to detect the absorbance of each well at 450 nm.

Construction of tumor-bearing mouse model

All BALB/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. All animal experiment procedures were carried out following the standard procedures approved by Shenzhen Institute of Advanced Technology Committee of the Chinese Academy of Sciences. The tumor model was

established by subcutaneously injecting 4T1 cells (2×10^6) into the back of mice. Animal studies were conducted when the tumor volume reaches approximately 100 mm^3 .

In Vivo Blood Circulation and Biodistribution

For circulation time measurement, first, 200 μL of AuNR@BSA was intravenously injected into the tail vein of BALB/c mice, and then 20 μL of blood was collected at 0.25, 2, 4, 6, 8, 12, 36, and 48 h, and diluted with 30 μL PBS to obtain 50 μL blood sample. About 400 μL concentrated HNO_3 (chromatographic grade) was added, the lid was tighten, and digested at 90°C for 2 h. After cooling to room temperature, 150 μL H_2O_2 (chromatographic grade) was slowly dropped in, and then heated to 90°C for 1 h without cover. In the end the solution was diluted to 5 mL by ultrapure water. The concentration of Au ion was measured by ICG-OEC after passing through 0.44 mm nylon syringe filter.

For biodistribution measure, about 200 μL AuNR@BSA was intravenously injected into the tail vein of BALB/c mice. After 24 h, the mouse was scarified and the heart, liver, spleen, lung, and kidney were taken and dried in an oven at 80°C . Before digestion, each organ was weighed, 800 μL concentrated HNO_3 (GR) was added, and heated to 90°C for 2 h. After cooling to room temperature, 200 μL H_2O_2 (GR) was slowly added dropwise, heated at 90°C for 1 h, and then the solution was diluted to 10 mL by ultrapure water. Finally, concentration of Au ion was measured by ICG-OEC after passing through 0.44 mm nylon syringe filter.

Photothermal treatment efficiency

In order to evaluate the thermal therapeutic effect of AuNR@BSA, the tumor-bearing mice with 4T1 tumors were randomly divided into four groups with the tumor volume about 100 mm^3 : (1) AuNR@BSA, (2) AuNR@BSA + Laser; (3) Only Laser (4) Blank control. An infrared thermal imager was used to record the infrared thermal image of the tumor site. The tumor volume and body weight of mice were recorded before and after treatment, respectively. The tumor volume can be calculated according to the normal equation ($\text{volume} = \text{width}^2 \times \text{length}/2$). Two weeks later, the mice were sacrificed, and the tumors were isolated.

Date Analysis

SPSS 16.0 statistical software was used for data analysis. The measurement data were expressed as mean $\pm$ d, the comparison between groups was done by analysis of variance, and the comparison of count data was performed by chi-square test. $P < 0.05$ was considered statistically significant.

Results And Discussion

Synthesis and Characterization of AuNR@CTAB

It was found that the smaller gold nanorods, the better pharmacokinetics and lower cytotoxicity [16]. However, the SPR absorption peak of gold nanorods is highly related to the aspect ratio, the larger the aspect ratio the longer the SPR peak. In order to synthesize AuNR with large aspect ratio in the meanwhile keep the size as small as possible, the synthesis parameters of the surfactant concentration, the pH of the growth solution, and the reducing agent concentration were optimized. The $\text{NaBH}_4(\text{aq})$ is a kind of strong reducing agent that forms Au nucleus through LaMer burst nucleation, followed by rapid random attachment of Au ions and intra-particle maturation [17]. As the mole quantity of NaBH_4 increasing, the maximum absorption peak of gold nanorods undergoes a blue shift from 1223 nm to 865 nm (Fig. 1C). The pH of the growth solution is also a key parameter to control the growth of gold nanorods, with is adjusted via the amount of hydrochloric acid [18]. The maximum absorption peak of gold nanorods was found to be gradually red-shifted from 871 nm to 1070 nm while increasing the quantity of hydrochloric acid (Fig. 1D). Furthermore, Ag^+ is considered to be able to control the growth direction of gold nanorods, the lower the Ag^+ concentration the longer the SPR peak could be realized (Fig. 1E). In the end, the gold nanorods synthesized at present work are in wine-red, as shown in Fig. 1B. Therefore, considering the synthesis efficiency of gold nanorods and the availability of lasers, we chose gold nanorods with a maximum absorption peak at 1064 nm for photothermal treatment of tumors.

Synthesis and Characterization of AuNR@BSA

Cetyltrimethylammonium bromide (CTAB) is the most widely used compound for the precise synthesis of gold nanorods with different lengths and aspect ratios. However, CTAB has significant cytotoxicity when the concentration is higher than 1–10 μM , and its application in biomedicine has been greatly restricted [19]. In addition to this problem, the colloidal stability of CTAB-stabilized gold nanorods in aqueous solution is affected by temperature changes, and it is easy to crystallize at low temperatures [20]. Given reducing the cytotoxicity of CTAB and improving its stability, many programs have been proposed to replace CTAB in the synthesis or functionally replace the CTAB of gold nanorods. Using polymers, peptides, surfactants, and lipids to modify nanoparticle surface, most of these strategies use thiolated molecules or electrostatic interaction forces to bind to the gold surface [21]. In this case, protein is a promising type of multifunctional coating, with the advantages of colloidal stability, biocompatibility, and further functionalization. It has various chemical functions and a determined nucleic acid structure, which can be targeted modified to target specific antigens. Among them, bovine serum albumin can completely remove CTAB, which is used as a shape directing agent to synthesize gold nanorods. Therefore, protein-coated nanorods are suitable for biological applications, where cationic surfactants must be avoided. Simultaneously, the colloidal stability of the system is significantly improved. The protocol is suitable for gold nanoparticles with a large aspect ratio and size range, and its main absorption frequency covers the visible light and near-infrared spectral range from 600 to 1100 nm. These colloidally stable and surfactant-free bovine serum albumin-coated gold nanorods can be applied in various plasmon and biomedical applications [22].

The CTAB and BSA wrapped gold nanorods were displayed as cartoon in Fig. 2A. The maximum absorption peak of AuNR@BSA is around 1064 nm, which is about 30 nm red-shifted compared to that of

AuNR@CTAB (Fig. 2B). The morphology of AuNR@BSA and AuNR@CTAB were characterized by transmission electron microscopy (TEM) as shown in Fig. 2D. The dynamic light scattering (DLS) was also applied for the AuNR@CTAB and AuNR@BSA. Two peaks could be clearly found from DLS intensity, one with hydrodynamic size about $3.10 (\pm 0.85)$ nm and the other about $57.45 (\pm 24.22)$ nm for AuNR@CTAB. However, in the case of AuNR@BSA, the peaks shift to $8.64 (\pm 3.80)$ nm and $89.24 (\pm 42.24)$ nm. We could find that the shape of AuNR remains similar after coating with BSA, except the head becomes slightly rounded (Fig. 2C, 2D). For in vivo therapy applications, the critical size of nanoparticles is limited to below 100 nm [23]. Beyond this size, the ability of nanoparticles to penetrate tumors will be limited, therefore, the presented gold nanorods would be an ideal tumor therapy candidate.

In vitro photothermal effect of gold nanorods

The 1064 nm diode laser as the most economic NIR-II light source available is considered to be the best option for photothermal therapy. Therefore, the ideal NIR-II gold nanopatform for efficient photothermal therapy must be characterized as strong SPR absorption at 1064 nm, high photothermal efficiency, and excellent photothermal stability. To investigate the photothermal effect of the prepared AuNR@BSA, PBS and different OD (= 0.5, 1, 1.5, 2) of AuNR@BSA were excited at 1064 nm with light intensity from 0.35 to 1 W/cm^2 for 30 min. A temperature imager was used to record the temperature changes every 5 minutes, as shown in Fig. 3A. The photothermal effect of AuNR@BSA and AuNR@CTAB with the same absorbance (OD = 1) is significantly higher than that of PBS. We could find that the temperature rose up rapidly in the first 5 min and then remained at about 90°C for the rest of time, as illustrated Fig. 3A. The photothermal induced temperature increase for PBS mostly caused by the overtone absorption of water at 1064 nm. The maximum temperature as a function of light intensity is illustrated in Fig. 3B. It was found that the photon induced thermal temperature of AuNR@CTAB and AuNR@BSA at absorbance about 1 is linear proportional to light intensity. The slope is much steeper for AuNR@CTAB and AuNR@BSA than that of PBS. In addition, Fig. 3C shows that under the same irradiation conditions (1064 nm, 0.75 W/cm^2), the maximum photothermal temperature is dramatically increased as the absorbance of both AuNR increasing. The photothermal properties of BSA coated AuNR is bit better than that of CTAB coated. It was also found that the maximum photothermal temperature of AuNR@BSA (OD = 1, 61.1°C) did not change much within three irradiation cycles (0.5 W/cm^2 , 10 min), indicating the excellent photothermal stability of the prepared AuNR@BSA (Fig. 3D). Figure 3E displays the photothermal temperature image of AuNR@CTAB (OD = 1), AuNR@BSA (OD = 1) and PBS under laser irradiation for 10 minutes. The maximum temperature of PBS is 40.9°C , while the maximum temperature of the AuNR is as high as 89.3°C . The above results prove that the presented AuNR@BSA possesses appropriate absorption characteristics, photothermal conversion efficiency and photostability in the NIR-II range and is expected to be an excellent photothermal therapeutic agent.

In vitro cytotoxicity and photothermal toxicity of gold nanorods

The CCK-8 analysis was applied to quantify the cytotoxicity of AuNR@CTAB and AuNR@BSA on 4T1 cells with and the prepared AuNR at different absorbance incubated for 24 h. In the case of without laser irradiation, The AuNR@CTAB at with low concentration (absorbance about 0.05) could dramatically inhibit the cell viability, hences the biological application of which is greatly restricted. However, in the case of AuNR@BSA, even that absorbance as high as 1, the cell viability is still acceptable about 84.78%, exhibits excellent biological application prospects (Fig. 4A). Encouraged by the promising stability and high photothermal conversion efficiency of AuNR@BSA, the photothermal toxicity was conducted on 4T1 tumor cells in vitro with light intensity about 0.75 W/cm^2 for 10 min. Significant photothermal toxicity was found at absorbance around 1 with cell survival rate about 20%, however about 100% cell survivivity was found for non radiation case (Fig. 4B). These in vitro results indicate that AuNR@BSA photothermal treatment in the NIR-II region could effectively kill cancer cells within 10 min.

In vivo biodistribution studies

Blood circulation time is essential for successful nanoparticle-based targeted drug delivery and anti-cancer therapy because it guides future application of nanocarriers [24]. The blood circulation time of AuNR@BSA was monitored by the Au concentration via ICG-OEC, which is about 1.5 hour (half-life) (Fig. 5A). The in vivo biodistribution of AuNR@BSA was also measured by the Au concentration in different organs. As shown in Fig. 5B, AuNR@BSA were highly accumulated in liver and spleen after 24 hours intravenous injection due to their strong phagocytosis as an organ of the reticuloendothelial system (RES) [25, 26]. These results show that AuNR@BSA can effectively accumulate in liver and spleen. AuNR@BSA has potential applications in liver and spleen-related diseases.

In vivo near-infrared photothermal treatment of gold nanorods

The excellent photothermal performance of AuNR@BSA encourage us to persue the in vivo photothermal therapy on tumor-bearing BALB/c mice. AuNR@BSA was first in situ injected into the tumor and then 10 min later the light was introduced for photothermal therapy (1064 nm , 0.75 mW/cm^2). The in vivo temperature variation was monitored by a temperature imager. The temperature changes before and after treatment were depicted in Fig. 7D. The temperature of tumor site was about 63.9°C within 10 min of irradiation and maintained small fluctuations at this temperature (Fig. 7D). In contrast, there is no observable temperature change for the control group in the same irradiation condition (AuNR@BSA group, PBS group, laser group). After photothermal therapy, the tumor volume and body weight of mice were monitored every two days for 14 days. As presented in Fig. 7A and 7B, the tumor of AuNR@BSA_laser group was completely inhibited and left as burned scabs on location of original tumor site, while that of the control groups grew relatively fast without any control (AuNR@BSA group, PBS group, laser group). The scabs are the burned skin, which directly prove that the PPT process could cause excess local heat on the tumor site with injected AuNR@BSA. The photothermal effect seems to be very fast, because we often observe that solid tumors in AuNR@BSA + laser group decrease rapidly two days after treatment. To

further capture the tumor changes after treatment, at the end of observation the 14-days, the mice were sacrificed and the tumors were isolated. As shown in Fig. 7C, tumor size of the AuNR@BSA_laser treatment group was totally suppressed, while all the control groups were found to be uncontrolled growth.

In addition, TEM was taken for the isolated tumor after photothermal treatment and the untreated tumor. The AuNR could be observed in the tumor tissue after photothermal treatment. It was proved time that gold nanorods still have excellent photostability at tumor tissue level (Fig. 6).

From figure neither the material injection group (only AuNR) nor the laser irradiation group (only Laser) showed tumor necrosis characteristics. 7C) manifested that the the best therapy (AuNR@BSA + laser) was significantly the smallest, proving the excellent tumor suppressor effect. Digital images of mice from group (AuNR@BSA + laser) showed that tumors did not continue to grow after 14-day treatment (Fig. 7E). Therefore, considering the ideal therapeutic ability and no obvious cytotoxicity, AuNR@BSA for near-infrared second zone laser irradiation therapy is an ideal candidate for light-triggered PTT in vivo. This clearly shows that simple hyperthermia using current NR-II therapy can effectively inhibit tumor growth.

Conclusion

Here, we synthesized a nano-platform of AuNR@BSA with the maximum SPR absorption peak in the second near-infrared window for photothermal therapy, as their outstanding photothermal properties, biocompatibility, and biodegradability. The biocompatibility of the reported AuNR was significantly improved by coating with bovine serum albumin, furthermore, the photothermal properties were not affected. The in vivo biodistribution of the intravenously injected AuNR@BSA indicates that large amounts of AuNR accumulated in the liver and spleen. At the tumor tissue level, the AuNR@BSA were observed inside or among tumor cells, and the photostability of the in vivo injected AuNR suggests that once upon injection, several phototreatment might be applied on the threpy outcome. Excellent photothermal conversion was observed even under low light irradiation, and sufficient high temperature on the tumors with NIR-II irradiation could completely inhibit tumor's growth. The AuNR@BSA nano platform combined with other treatment strategies, such as immunotherapy and chemotherapy, will be important for developing a useful tool for personalized, safe, and effective treatment.

Declarations

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Availability of data and materials

The data set used and/or analyzed in this study can be obtained from the corresponding author upon reasonable request. All data generated or analyzed during this study is included in this published article.

Authors' Contributions

Qinchao Sun conceived and designed the experiments. Shubi Zhao performed the experiments. Shubi Zhao analyzed the data. Shubi Zhao and Yiqun Luo wrote the manuscript. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare that they have no competing interests.

References

1. Kumar D, Soni RK, Ghai DP, Pulsed photoacoustic and photothermal response of gold nanoparticles, *Nanotechnology* 31(2020) 3570. <https://doi.org/10.1088/1361-6528/ab47ae> 4
2. Boisselier E, Astruc D (2009) Gold nanoparticles in nanomedicine: preparations, imaging, diagnostics, therapies and toxicity. *Chem Soc Rev* 38:1759–1782. <https://doi.org/10.1039/b806051g>
3. Haine AT, Niidome T, Gold Nanorods as Nanodevices for Bioimaging, Photothermal Therapeutics, and Delivery D, *Chemical & pharmaceutical bulletin*. 65(2017) 625–628. <https://doi.org/10.1248/cpb.c17-00102>
4. De Matteis V, Cascione M, Toma CC, Rinaldi R (2019) Engineered Gold Nanoshells Killing Tumor Cells: New Perspectives. *Curr Pharm Des* 25:1477–1489. <https://doi.org/10.2174/1381612825666190618155127>
5. Cobley CM, Au L, Chen J, Xia Y (2010) Targeting gold nanocages to cancer cells for photothermal destruction and drug delivery. *Expert Opin Drug Deliv* 7:577–587. <https://doi.org/10.1517/17425240903571614>
6. Chang SS, Shih CW, Chen CD, Lai WC, Wang C (1998) The shape transition of gold nanorods. *Langmuir* 15:701–709. <https://doi.org/10.1021/la980929l>
7. Smith AM, Mancini MC, Nie S (2009) Bioimaging: second window for in vivo imaging. *Nat Nanotechnol* 4:710–711. <https://doi.org/10.1038/nnano.2009.326>
8. Jana NR, Gearheart L, Murphy CJ (2001) Seed-Mediated Growth Approach for Shape-Controlled Synthesis of Spheroidal and Rod-like Gold Nanoparticles Using a Surfactant Template. *Advanced Materials* 13:1389–1393. [https://doi.org/10.1002/1521-4095\(200109\)13:18%3C1389::AID-ADMA1389%3E3.0.CO;2-F](https://doi.org/10.1002/1521-4095(200109)13:18%3C1389::AID-ADMA1389%3E3.0.CO;2-F)
9. Nikoobakht B, El-Sayed MA, Preparation and Growth Mechanism of Gold Nanorods (NRs) Using Seed-Mediated Growth Method, *Chemistry of Materials*. 15(2003)1957–1962. <https://doi.org/10.1021/cm020732l>

10. Mehdizadeh S, Pandesh A, Shakeri-Zadeh SK, Kamrava M, Habib-Agahi M, Farhadi M, Pishghadam A, Ahmadi S, Arami Y, Fedutik (2014) The effects of folate-conjugated gold nanorods in combination with plasmonic photothermal therapy on mouth epidermal carcinoma cells. *Lasers Med Sci* 29:939–948. <https://doi.org/10.1007/s10103-013-1414-2>
11. Li L, Lu M, Zhao Z, Lei F, Zhang (2018) An Efficient 1064 nm NIR-II Excitation Fluorescent Molecular Dye for Deep-Tissue High-Resolution Dynamic Bioimaging. *Angew Chem Int Ed Engl* 57:7483–7487. <https://doi.org/10.1002/anie.201801226>
12. Ding F, Chen Z, Kim WY, Sharma A, Li CL, Ouyang QY, Zhu H, Yang G, Sun Y, Kim JS (2019) A nano-cocktail of an NIR-II emissive fluorophore and organoplatinum(ii) metallacycle for efficient cancer imaging and therapy. *Chem Sci* 10:7023–7028. <https://doi.org/10.1039/c9sc02466b>
13. Ma Y, Zhang Y, Li X, Zhao Y, Li M, Jiang W, Tang X, Dou J, Lu L, Wang F, Wang Y (2019) Near-Infrared II Phototherapy Induces Deep Tissue Immunogenic Cell Death and Potentiates Cancer Immunotherapy. *ACS Nano* 13:11967–11980. <https://doi.org/10.1021/acsnano.9b06040>
14. Wang J, Wu C, Qin X, Huang Y, Zhang J, Chen T, Wang Y, Ding Y, Yao Y (2021) NIR-II light triggered nitric oxide release nanoplatform combined chemo-photothermal therapy for overcoming multidrug resistant cancer. *J Mater Chem B* 9:1698–1706. <https://doi.org/10.1039/d0tb02626c>
15. Ahmad R, Fu J, He N, Li S (2016) Advanced Gold Nanomaterials for Photothermal Therapy of Cancer. *J Nanosci Nanotechnol* 16:67–80. <https://doi.org/10.1166/jnn.2016.10770>
16. Li Z, Tang S, Wang BK, Li Y, Huang H, Wang H, Li P, Li C, Chu PK, Yu. X, Metabolizable Small Gold Nanorods: Size-dependent Cytotoxicity, Cell Uptake and In Vivo Biodistribution, *ACS Biomater Sci Eng.* 2(2016)789–797. <https://doi.org/10.1021/acsbiomaterials.5b00538>
17. Liu T, Shi S, Liang C, Shen S, Cheng L, Wang C, Song X, Goel S, Barnhart TE, Cai W, Liu Z, Iron oxide decorated MoS₂ nanosheets with double PEGylation for chelator-free radiolabeling and multimodal imaging guided photothermal therapy, *ACS Nano.* 9(2015)950 – 60. <https://doi.org/10.1021/nn506757x>
18. Thanh NT, Maclean N, Mahiddine S (2014) Mechanisms of nucleation and growth of nanoparticles in solution. *Chem Rev* 114:7610–7630. <https://doi.org/10.1021/cr400544s>
19. Chen YS, Zhao Y, Yoon SJ, Gambhir SS, Emelianov S (2019) Miniature gold nanorods for photoacoustic molecular imaging in the second near-infrared optical window. *Nat Nanotechnol* 14:465–472. <https://doi.org/10.1038/s41565-019-0392-3>
20. Kah JC, Chen J, Zubieta A, Hamad-Schifferli K (2012) Exploiting the protein corona around gold nanorods for loading and triggered release. *ACS Nano* 6:6730–6740. <https://doi.org/10.1021/nn301389c>
21. Ray PC, Yu H, Fu PP, Toxicity and Environmental Risks of Nanomaterials: Challenges and Future Needs, *J Environ Sci Health Part C: Environ Carcinog Ecotoxicol Rev* 27(2009)1 – 35. <https://doi.org/10.1080/10590500802708267>
22. Tebbe M, Kuttner C, Männel M, Fery A, Chanana M (2015) Colloidally stable and surfactant-free protein-coated gold nanorods in biological media. *ACS Appl Mater Interfaces* 7:5984–5991.

<https://doi.org/10.1021/acsami.5b00335>

23. Perrault SD, Walkey C, Jennings T, Fischer HC, Chan WC (2009) Mediating tumor targeting efficiency of nanoparticles through design. *Nano Lett* 9:1909–1915. <https://doi.org/10.1021/nl900031y>
24. Zhang ZJ, Wang J, Nie X, Wen T, Ji YL, Wu X, Zhao YL, Chen CY (2014) Near Infrared Laser-Induced Targeted Cancer Therapy Using Thermoresponsive Polymer Encapsulated Gold Nanorods. *J Am Chem Soc* 136:7317–7326. <https://doi.org/10.1021/ja412735p>
25. Qhattal HSS, Hye T, Alali A, Liu XL, Length HP (2014) Grafting Density, and Surface Poly(Ethylene Glycol) Coating Influence in Vivo Circulation and Tumor Targeting of Hyaluronan-Grafted Liposomes. *ACS Nano* 8:5423–5440. <https://doi.org/10.1021/nn405839n>
26. You Q, Sun Q, Yu M, Wang J, Wang S, Liu L, Cheng Y, Wang Y, Song Y, Tan F, Li N, BSA-Bioinspired Gadolinium Hybrid-Functionalized Hollow Gold Nanoshells for NIRF/PA/CT/MR Quadmodal Diagnostic Imaging-Guided Photothermal/Photodynamic Cancer Therapy, *ACS Appl Mater Interfaces* 9(2017)40017–40030. <https://doi.org/10.1021/acsami.7b11926>

Figures

Figure 1

The photoproperties of AuNR@CTAB in different synthesis conditions [1]. (A) Preparation of AuNR@CTAB. (B) Image of CTAB-coated gold nanorods (AuNR@CTAB), (C) UV-vis spectra of prepared AuNR@CTAB with varying NaBH_4 concentration, (D) UV-vis spectra of prepared AuNR@CTAB with

varying HCl concentration (E) UV-vis spectra of prepared AuNR@CTAB with varying AgNO3 concentration

Figure 2

Characterization of AuNR@BSA and AuNR@CTAB. (A) Preparation of AuNR@BSA. (B) UV-Vis images of AuNR@CTAB (L) and AuNR@BSA (R). (C) The size of AuNR@CTAB and AuNR@BSA. (D) TEM images of AuNR@CTAB and AuNR@BSA.

Figure 3

In vitro evaluation of the PTT effect. (A) NIR-triggered temperature increase of AuNR@BSA and AuNR@CTAB with same SPR absorbance (OD=1) for 30 min laser irradiation (1064 nm, 0.75 W/cm²). (B) NIR-triggered temperature increase of PBS, AuNR@CTAB (OD=1), and AuNR@BSA (OD=1) for 30 min laser irradiation (1064 nm, 0.35 to 1 W/cm²). (C) NIR-triggered temperature increase of AuNR@BSA and AuNR@CTAB with different absorbance for 10 min laser irradiation (1064 nm, 0.75 W/cm²). (D)

Photothermal conversion of AuNR@BSA (OD=1) for three repeated irradiation cycles (1064 nm, 0. 5 W/cm2). (E) Thermal image of PBS, AuNR@CTAB (OD = 1), AuNR@BSA (OD = 1), irradiated with NIR laser (1064 nm, 0.75W/cm2) at a determined time interval (5 and 10 minutes).

Figure 4

In vitro cytotoxicity and photothermal toxicity of gold nanorods. (A) Cell viability of 4T1 cells incubated with various concentrations of AuNR@CTAB and AuNR@BSA. (B) Cell viability of 4T1 cells incubated with various concentrations of AuNR@BSA without (a) or with (b) NIR laser irradiation (1064 nm, 0.75 W/cm2, 10 min). (mean $\pm$ SD, n = 3).

Figure 5

Study on the biodistribution of AuNR@BSA in blood and organs in vivo. (A) In Vivo Blood Circulation. (B) In Vivo Biodistribution of various organs.

Figure 6

Transmission electron micrograph of gold nanorods in tumor tissue (A) Treated tumor. (B) Untreated tumor.

Figure 7

Photothermal treatment efficiency. (A) Tumor volume as a function of time under different treatment condition. (B) Body weight changes of mice bearing tumors as a function of time, post-treatment for various groups. (C) Photographs of tumor tissues in different groups after 14-day treatment. (D) Infrared thermographic images of tumor-bearing mice exposed to a 1064 nm laser (0.75 W/cm², 10 min) for determined time intervals 10 min post intratumoral injection of AuNR@BSA. (E) Images of mice from AuNR@BSA_laser group after treatment. (mean $\pm$ SD, n = 2).