Fabrication of gold-coated silica nanorods for photothermal therapy based on phase separation of polymer blends

Yuanhao Jiang¹, Yang Li¹, Xinxin Fu¹, Yushuang Cui¹,², Changsheng Yuan¹,², Haixiong Ge¹,²,*

¹Department of Materials Science and Engineering, College of Engineering and Applied Sciences, Nanjing University, Nanjing 210093, China
²Collaborative Innovation Center of Advanced Microstructures, Nanjing 210093, China

*Corresponding author e-mail: haixiong@nju.edu.cn

Abstract. In this study, we demonstrated a novel on-substrate approach to fabricate gold-coated silica nanorods (GSNRs) with potential application in cancer hyperthermia therapy. Based on the circular nanodomains formed by phase separation of the polymer blend of polyphenylsilsequioxane (PPSQ) and polystyrene (PS), GSNRs were facilely obtained by means of conventional fabrication methods including reactive ion etching (RIE), e-beam evaporation deposition and lift-off. And it was simple and reliable to adjust the geometry of GSNRs by controlling the parameters of phase separation process and the thickness of the SiO₂ layer. Surface modification of GSNRs with polyethylene glycol (PEG) was performed to increase their stability and biocompatibility in the aqueous solution. Due to the localized surface plasmon resonance (LSPR) of gold, there was an absorbance peak in near-infrared (NIR) region. The significant enhancement of heating effect indicated that GSNRs could rapidly and efficiently convert the laser energy into heat under 808 nm laser irradiation. In addition, the positive results of cytotoxicity test indicated that GSNRs were suitable for further in vivo applications.

1. Introduction
Cancer is one of the major threats to human health. Currently, the main choices to treat cancers are still traditional methods, such as chemotherapy, surgery, radiation therapy, etc.[1] Unfortunately, these methods are restricted due to the side effects. In recent years, plasmonic photothermal therapy (PPTT) [2-4] has attracted a great deal of attention because of its minimal invasiveness and precise selectivity. In this PPTT protocol, plasmonic nanomaterials are delivered to the tumor tissue, where they absorb light and convert it into heat, producing a local hyperthermia to kill the tumor cells. Gold nanoparticles are one of the most promising PPTT materials because of their strong optical absorption properties, tunable extinction spectra, and ease of surface modification. What’s more, due to these properties, gold nanoparticles are widely used in many biomedical applications, such as cellular imaging [2, 5], biosensing [6, 7], cancer therapy [8, 9], and drug delivery [10, 11].

In the case of PPTT, near-infrared (NIR, 700-1200 nm) laser is being widely used as the light source because it can reach several centimeters in biological tissues due to its relatively weak...
absorption by water and hemoglobin. [12] Owing to the easily tunable localized surface plasmon resonance (LSPR) of gold, it is possible to adjust the extinction spectra of gold nanoparticles into NIR region by changing their shape and size. Upon exposure to NIR laser excitation, ~10-200 nm gold nanoparticles can support LSPR which is coherently localized oscillations of free conduction band electrons. [13] Much work has been made to synthesize nanoparticles with LSPR bands in the NIR region, for instance nanorods [14], nanospheres [15], nanoshells [16, 17], and nanocages [18]. In particular, gold nanorods (AuNRs) are considered as excellent photothermal agents for PPTT as they display a strong absorption in NIR region and efficient photothermal conversion.

In general, gold nanorods are prepared by chemical synthesis, such as wet chemical seed-mediated synthesis [19] and electrochemical synthesis [20]. In this study, we demonstrated a simple and effective on-substrate approach to fabricate GSNRs by means of combining bottom-up phase separation approach [21-23] and top-down fabrication methods including reactive ion etching (RIE), e-beam evaporation deposition and lift-off. Polyphenylsilsesquioxane (PPSQ) and polystyrene (PS) were utilized as two components of the polymer blend for phase separation approach to form circular nanopatterns. The nanopattern was transformed into SiO$_2$ nanopillars via a multiple-step etching process. Various-aspect-ratio nanopillars with different diameters can be obtained by tuning the parameters of phase separation and the thickness of the SiO$_2$ layer. Gold was then deposited onto the SiO$_2$ nanopillars by e-beam evaporation. The GSNRs were released from the substrate after lift-off process. The Vis–NIR extinction spectra of GSNRs solution displayed an LSPR peak in NIR region. The significantly enhancement of heating effect and low cytotoxicity indicated that the GSNRs were suitable for further in vivo applications.

2. Experiment section

![Figure 1](image_url) Figure 1. Schematics of fabrication process of GSNRs. (a) Polymer blend/SU-8/SiO$_2$/PVP multilayer structure; (b) PS component etching by O$_2$ RIE; (c) removing residual PPSQ layer by fluorine-based RIE; (d) SU-8, (e) SiO$_2$ and (f) PVP layer etching by RIE; (g) deposition of 20-nm-thick gold at a controlled angle $\theta$ from two sides by e-beam evaporation; (h) forming GSNRs after lift-off process.
2.1. Materials
All the chemicals, including toluene and other reagents, are commercially available and used without further purification. PS (MW = 100 000) was obtained from Sigma-Aldrich. PPSQ was purchased from Gelest, Inc. SU-8 2150 was purchased from MicroChem. mPEG-SH (MW = 5 000) was purchased from HUATENG PHARMA. Polyvinylpyrrolidone (MW = 3 500) was purchased from Energy Chemical. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Fabrication of GSNRs.
Schematics of the fabrication process of GSNRs is shown in Figure 1. A 90-nm thick PVP layer was first spin-coated on silicon substrates as a sacrificial layer. Then, a SiO₂ layer was deposited on top of the PVP layer by plasma-enhanced chemical vapor deposition (PECVD) at 30 °C. Subsequently, a 150-nm thick SU-8 photoresist layer was spin-coated on top of SiO₂ layer and then cross-linked via a UV exposure and postbaking process. After an O₂ RIE treatment of SU-8, the PPSQ/PS binary polymer blends were spin-coated onto it, as shown in Figure 1a. The PS component was removed by an O₂ RIE process, as shown in Figure 1b. The fluorine-based RIE was used to eliminate the silica-like residual layer, as shown in Figure 1c. The circular nanodomains were then transferred into the SU-8 layer and SiO₂ layer by the O₂ RIE and fluorine-based RIE, respectively, as shown in Figure 1d and e. After that, the O₂ RIE was used toetch the PVP layer and remove the residual SU-8 layer, as shown in Figure 1f. Afterwards, 20-nm thick gold was deposited at a controlled angle θ of 30 ° from two sides, as shown in Figure 1g. The GSNRs were detached from the substrate after the lift-off process in deionized (DI) water with ultrasonic cleaning, as shown in Figure 1h. Subsequently, the GSNRs were harvested by centrifugation for 15 min at 6000g and were then redispersed in DI water.

2.3. Surface modification of GSNRs.
Modification with PEG is an effective approach to increase stability and biocompatibility of the GSNRs in the aqueous solution. PEGylation of the GSNRs was performed with m-PEG-SH via covalent bonding between mercap groups and gold atoms. In brief, 100 mg m-PEG-SH (Mw 5 kDa) was added into the aforementioned GSNRs solution, keeping stirring overnight at room temperature. The solution was purified by centrifugation for 15 min at 6000 g and the GSNRs were then redispersed in DI water.

2.4. In vitro cytotoxicity assay (MTT assay).
Cytotoxicity of GSNRs was tested via a MTT colorimetric assay. Briefly, HeLa cells were seeded in 96-well plates (5×10³ cells/well) and incubated overnight at 37°C in 5% CO₂ atmosphere. After that, the cells were washed and incubated with 200 μL fresh culture medium containing various concentrations of GSNRs, and 20 μL of MTT (5 mg/mL) was added into each well after 24 h. All concentrations were tested in five replicates. After another 4 h of incubation, the medium containing the MTT was removed, and the formazan crystals were dissolved after the addition of 150 μL of dimethyl sulfoxide (DMSO) for 0.5 h. And the absorbance of each well at 490 nm was measured by an Infinite M200 Pro plate reader (Tecan, Switzerland).

2.5. Photothermal Heating of GSNRs Solutions.
The photothermal effect induced by GSNRs was investigated by irradiating the solutions with the 808 nm (1 W/cm²) laser. The temperatures were monitored by directly placing a thermocouple into the solutions and were recorded every 20s until 10 min. To ensure uniformity, the initial temperature of all solutions was 25 °C. Three independent experiments were performed as contrasts.

2.6. Reactive-Ion Etching (RIE).
All the RIE processes were performed on an inductively coupled plasma (ICP) system (CE-300I, ULVAC). An O₂-based plasma (10 sccm, 2 Pa pressure, 30 W power) was used to remove PS and etch
the SU-8 photoresist and PVP layer. A CHF$_3$/O$_2$-based plasma (20/20 sccm, 2 Pa pressure, 30 W power) was used to eliminate the silica-like residual layer. A CHF$_3$/CF$_4$-based plasma (10/30 sccm, 2 Pa pressure, 40 W power) was used to etch the SiO$_2$ layer.

2.7. Characterization.
All the SEM images were obtained using a field-emission scanning electron microscope (ZEISS ULTRA-55). The core shell structure of GSNRs was observed by a transmission electron microscope (FEI Tecnai F20). Surface plasmon resonance property was explored by UV–vis–NIR spectrophotometer (Shimadzu UV-3600).

**Figure 2.** (a) Top-view SEM images of SiO$_2$/PVP bilayer phase separation structures after RIE process at concentrations of 2 wt % and (b) 3 wt %. The weight ratio of PPSQ/PS was 1:1; (c) Cross-sectional view of SEM images of SiO$_2$/PVP bilayer nanopillars after RIE process, the average heights of SiO$_2$ nanopillars were 210 nm and (d) 320 nm; (e) Cross-sectional view of SEM image of SiO$_2$/PVP bilayer nanopillars after gold deposition; (f) Top-view SEM image of the GSNRs, the inset was the TEM image of the GSNRs.
3. Results and discussion

In this work, PPSQ/PS blends were chosen as phase separation systems due to their miscibility. The solutions of the polymer blends were prepared by dissolving PS and PPSQ into toluene at various weight ratios and concentrations. Since PPSQ and silica had a common composition of silicon, PPSQ would also be etched if it was used as the mask to etch silica directly, making it difficult to transfer the phase separated PPSQ structures to silica with a high aspect ratio. To solve the above issue, a pure organic film, SU-8 photoresist, was introduced underneath the PPSQ/PS polymer blend film as a transfer layer. SU-8 is a commonly used negative photoresist, which can form a highly cross-linked polymer network via a UV exposure and postbaking process. Its cross-linked film exhibits excellent chemical and thermal stability, mechanical properties, and high resistance to dissolving or swelling in the polymer blend solution during spin-coating. The water contact angles of PPSQ, PS and the SU-8 surface are 78°, 89° and 84°, respectively. But in the phase separation process, it is necessary to provide a neutral substrate for the lateral phase separation of polymer blend with similar surface tensions. So in this work, the SU-8 film surface was oxidized by a 40s O₂ RIE treatment to make it more hydrophilic, which could be proven by the decrease of water contact angle from 84° to 42° on SU-8 surface. As PPSQ and PS had similar surface tensions and differed from that of the treated SU-8 surface, the treated SU-8 substrate provided nonpreferential interactions to each component. After O₂ RIE, the PPSQ/PS phase separation morphologies were transformed into nanopillars, the isolated circular domains of the PPSQ phase were vertically penetrated and laterally distributed in the PS matrix.[23] Generally, the phase separated domains are impure because of the chain entanglement between the different polymer components. This means PPSQ is slightly mixed in the PS-rich phase and PS is slightly mixed in the PPSQ-rich phase. After O₂ RIE, PS was removed while the PPSQ remaining in the PS-rich phase formed a silica-like residual layer, which was eliminated by the following fluorine-based RIE. Afterwards, the circular nanodomains were transferred into the SU-8 layer, SiO₂ layer and PVP layer via a series of selective etching processes. By tuning the parameters of phase separation and thickness of the SiO₂ layer, we can fabricate various-aspect-ratio nanopillars structures with different diameters. Figure 2a and b show a top view of SiO₂/PVP bilayer phase separation structure after a multiple-step etching process at concentrations of 2 and 3 wt %, respectively, with a fixed PPSQ/PS weight ratio of 1:1. The inset shows the feature size distribution of nanopillars. The nanopillar diameter ranged from 51 to 235 nm with an average of 143 nm at a concentration of 2 wt %, while it ranged from 63 to 334 nm with an average of 213 nm at a concentration of 3 wt %. With the increase of concentration, there was a wider range of diameter distribution and it had more opportunity to form a continuous structure. Figure 2c and d show the cross-sectional view of SEM image of SiO₂/PVP bilayer nanopillars with a PPSQ/PS ratio of 1:1 and a concentration of 2 wt %. The average heights of SiO₂ nanopillars were 210 and 320 nm, respectively, which can be tuned by controlling the deposition time of SiO₂ layer. The PVP nanopillars were thinner than the SiO₂ nanopillars due to the lateral etching during O₂ RIE process. Figure 2e shows a cross-sectional view of SEM image of SiO₂/PVP bilayer nanopillars after gold deposition process. The average diameter and height of the gold-coated SiO₂ nanopillars increased to 165 nm and 250 nm, respectively. After lift-off process, the GSNRs were harvested by centrifugation and then redispersed in DI water. Figure 2f shows a top view of SEM image of the GSNRs. The inset shows a TEM image of the GSNRs. According to the different contrast between internal and surface, the dark part of indicated gold and the light part indicated SiO₂. It was clear that the gold deposited on the top is thicker than that on the sidewall and there was no gold at the bottom. However, not all the SiO₂ nanorods were completely coated with gold on the sidewall because gold was not uniformly deposited from all angles and the SiO₂ nanopillar might be blocked by the surrounding nanopillars during the deposition process. Considering that the size of nanorods should not be too large, GSNRs with an average diameter of 165 nm and a height of 250 nm were chosen and used in subsequent experiments.
Figure 3. Vis−NIR extinction spectra of the GSNRs.

The Vis−NIR extinction spectra of GSNRs was measured in DI water. As shown in Figure 3, it displayed an LSPR peak at 796 nm with a wide range of resonant wavelengths. The feature sizes of GSNRs were mainly distributed in 100-200 nm and the height of SiO$_2$ nanorods was 250 nm, so the aspect ratios were range from 1.2 to 2.5. In general, the Vis−NIR extinction spectra of gold nanorods has two absorbance peaks, transverse and longitudinal surface plasmon resonance, correlated with the shorter transverse axis and the longer longitudinal axis, respectively. However, there was only one wideband absorbance peak in our case. It could be attributed to two main reasons. On one hand, the final Vis−NIR extinction spectra was the combination of that of nanorods with different aspect ratios. On the other hand, as aforementioned, the SiO$_2$ nanorods were not completely coated with gold because gold was only deposited from two sides and the SiO$_2$ nanopillar might be blocked by the surrounding nanopillars during the deposition process. Despite of that, there was no problem for the photothermal conversion of GSNRs.

Figure 4. Temperature increase induced by PPTT heating of GSNRs solutions under 808 nm laser irradiation. High concentration solution (blue) shows the largest temperature increase to 51.8 °C at 10 min, followed by low concentration solution (red) at 44.1 °C at 10 min, and DI water (black) at 26.7 °C at 10 min. The initial temperature of all solutions was 25 °C.
The photo thermal effects of the GSNRs were measured directly in aqueous solutions under 808 nm laser irradiation (1 W/cm$^2$) for 10 min. The temperature increase of high concentration solution (400 μg/mL), low concentration solution (200 μg/mL), and DI water were measured, respectively. As shown in Figure 4, after a same exposure time of 10 minutes, the temperature of DI water was found to reach a maximum of 26.7 °C, while it reached 44.1 °C and 51.8 °C in the low concentration solution and high concentration solution respectively. In comparison, the DI water showed a minimal thermal response while the GSNRs solutions displayed strong thermal response. It was obviously shown that higher concentration of the GSNRs solution provided a significantly larger enhancement of heating effect, the temperature enhancement of the high concentration solution was 1.4 times of that of the low concentration solution. The concentration dependent temperature enhancement indicated that GSNRs could rapidly and efficiently convert the laser energy into heat under 808 nm laser irradiation. In particular, after 260s of NIR laser irradiation, the temperature in the high concentration solution increased to 46 °C, which was sufficient to induce hyperthermia. [24]

![Figure 5. Cytotoxicity profiles of HeLa cells post 24 h incubation with GSNRs via MTT assay.](image)

What’s more, in order to evaluate the feasibility of further in vivo applications, the cytotoxicity test of the GSNRs was performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Negligible cytotoxic effects on cell viability were observed after these cells were incubated with GSNRs at various concentrations ranging from 12.5 to 200 μg/mL for 24 h. After 24 h, a slight reduction in cell viability (9.8%) was observed at the highest concentration (200μg/mL), indicating that the GSNRs had low cytotoxicity and were suitable for in vivo applications.

4. Conclusion
In summary, this work demonstrated a simple and low-cost approach to fabricate GSNRs by phase separation and traditional fabrication methods. It was capable of fabricating various-aspect-ratio nanopillars with different diameters by tuning the parameters of phase separation and thickness of the SiO$_2$ layer. The Vis−NIR extinction spectra of GSNRs solution displayed an LSPR peak in NIR region. Furthermore, the photothermal effect and cytotoxicity indicated that GSNRs were suitable for further in vivo applications.

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