Research article

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Smart NIR-light and pH responsive doxorubicin-loaded GNRs@SBA-15-SH nanocomposite for chemo-photothermal therapy of cancer

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Abstract: We designed novel biocompatible nanocomposite composed of gold nanorods coated with rod-like mesoporous silica SBA-15-SH particles, (GNRs@SBA-15-SH) as a new synergistic therapeutic device to deliver heat and drug to cancer cells for tumor eradication. For this purpose, doxorubicin (DOX) was loaded into GNRs@SBA-15-SH nanocomposites and studied their photothermal therapy, chemotherapy and the combined effect on the ablation of A549 cells *in vitro* using human lung cancer cells, A549. The results demonstrate the high photothermal efficacy of gold

nanorods loaded into the nanocomposite, the thermo-responsive properties of GNRs@SBA-15-SH, the high loading capacity of DOX into the GNRs@SBA-15-SH and its biocompatibility. Synergistic chemo-photothermal of the GNRs@SBA-15-SH/DOX nanocomposite in the eradication of cancer cells under laser irradiation (808 nm), demonstrates the high potential of therapeutic efficacy of this combined therapy over monotherapies.

Keywords: cancer therapy; chemo-photothermal therapy; doxorubicin; gold nanorods.

1 Introduction

Plasmonic photothermal therapy is a promising strategy to treat different cancer types [1–4]. In recent years, the development of novel nanomaterials as photothermal contrast agents including polymeric nanoparticles [5, 6], carbon-based nanomaterials [7, 8], metal nanoparticles [9–13] and semiconductor nanoparticles [14, 15] have received a great of attention. In this technique, the plasmonic nanoparticles due to their unique optical properties, their biocompatibility [16–18] and their localized surface plasmon resonance (LSPR) [19, 20] can be used to minimize the thermal damage to the nontarget tissues and to treatment of deep tumor tissues [1].

Gold nanoparticles (GNPs) with particular shapes such as cages [11], rods [10], shells [21], stars [22], spheres [23], cubes [24], etc. have received a great deal of attention for tumor photothermal therapy (PTT) due to their high optical adsorption coefficients in the near-infrared region (NIR) (650–900 nm), where the water and hemoglobin, the major absorbers of infrared and visible light, respectively, have minimal light absorption [25–30]. This spectral range is therefore called the “therapeutic window” or the “tissue optical window” [31].

Among the various PTT agents based on GNPs, gold nanorods (GNRs), have been extensively examined for

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cancer therapy because of their tunable LSPR to convert NIR light into heat, ease of preparation, biocompatibility and surface functionalization to allow for tumor targeting [32–34]. However, photothermal cancer therapy with GNRs alone has several drawbacks [35]. The structure of the nonporous GNRs show low loading capacities and low-efficiency of photothermal conversion because of the intensities of NIR light will gradually decrease as it crosses the body tissues to deeper tumors [36–39]. Thus, the heat converted from light is inefficient to kill cancer cells in deep tissues.

In addition, increasing laser irradiation intensity can cause thermal damage to the surrounding healthy tissues [40]. Cetyltrimethylammonium bromide (CTAB)-coated GNRs showed considerable cytotoxicity to human cells due to the highly cytotoxic property of unbound CTAB. Furthermore, CTAB-coated GNRs were frequently observed to aggregate affecting their unique optical properties and to have low uptake into cancer cells [16, 41]. This has limited the potential use of CTAB-coated GNRs in biomedical applications. Novel strategies are therefore needed to enhance the therapeutic efficacy of photothermal cancer therapy [42].

During the last decade, it has been shown that combining PTT with chemotherapy can be more effective than either of the two treatments alone. This is because hyperthermia was shown to result in faster drug release into tumor [43], and an enhancement of the antitumor activity of chemotherapeutic drugs such as doxorubicin and cisplatin [44]. By having these facts, GNRs coated with stimulus-responsive polymers, porous silica, DNA, proteins, and porous metal–organic framework with high loading capacities has been developed to evaluate the effect of chemo-photothermal therapy [40].

Recently, mesoporous silica nanoshell (MSN) has been explored as an appropriate coating material for GNRs for combining chemotherapy with photothermal therapy (chemo-photothermal therapy) to enhance the efficacy of anticancer agents [38, 45, 46]. However, the surface area and the pore size of GNR@mSiO₂ are generally in the range of 50–350 m²/g and 0.5–4.0 nm, respectively [38, 46, 47] which leads to a rather low drug loading capacity and sub-optimal therapeutic effects.

Among the various MSN, ordered hexagonal mesoporous silica materials (SBA-15) have evolved as a new generation nanoporous materials with high surface area (range of 700–1200 m²/g) with uniform pore sizes, hydrothermal stability, high surface area, shape selectivity, and large-pore volume [48, 49]. Due to their shape and internal morphological structure and pore size, they can be adapted [49–51] to load different biomolecules with a broad range of size and shape.

One of the most important features of the structure and chemistry of pure SBA-15 is the existence of silanol groups on their external surface which form very weak intermolecular hydrogen bonds with many drug molecules. Therefore, they are capable of releasing the attached drug in a sustained manner for a relatively long period of time. Owing to the unique regularity of the mesopores of SBA-15 with two-dimensional hexagonal structure, it's a valuable substrate to develop and modify with various organic functional groups on the internal surface of SBA-15 by silylation as mesoporous carriers for controlling release of entrapped drug [52–54].

In this study, we report the design of a novel nanocomposite of GNRs@SBA-15-SH/DOX to integrate PTT and chemotherapy. Coating GNRs with SBA-15 nanoparticles functionalized with mercaptosilane groups has enhanced both the photothermal activity of GNRs and the drug loading capacity. Doxorubicin (DOX) as chemotherapeutic agent was directly incorporated into the formed GNRs@SBA-15-SH. *In vitro* experiments in this study indicate the biocompatibility and the antitumor efficacy of GNR@SBA-15-SH/DOX using A549 lung cancer cells. Interestingly, our results showed that the synergistic effect of PTT and chemotherapy of nanocomposite in killing A549 lung cancer cells is because of the intracellular drug release activated by NIR light remotely.

2 Materials and methods

2.1 Materials

Cetyltrimethylammonium bromide (CTAB), sodium borohydride, L-ascorbic acid, silver nitrate, gold (III) chloride solution (HAuCl₄·3H₂O), [3-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide] (MTT), tetraethylorthosilicate (TEOS), (3-mercaptopropyl)trimethoxy-silane (MPTMS), and pluronic copolymer P123 (EO₂₀PO₇₀EO₂₀) with typical molecular weight 5800 were purchased from Sigma-Aldrich. Doxorubicin hydrochloride (DOX) was obtained from Fenghua Lianbo. Co (Peking, China). Phosphate-buffered saline (PBS) solution was prepared by adding NaCl, KCl, Na₂HPO₄ and KH₂PO₄ in deionized water in concentrations of 8.13, 0.2, 1.44, and 0.2 g/L, respectively. Deionized water was used in all experiments.

2.2 Synthesis of GNRs

GNRs were synthesized following an established seed-mediated growth method [55]. Briefly, a seed solution was obtained by adding ice-cold freshly prepared NaBH₄ solution (600 μL, 10 mM) into an aqueous mixture solution of HAuCl₄ (250 μL, 10 mM) and CTAB (7 mL, 100 mM). The solution was mixed by vigorous stirring for 2 min and the obtained brownish yellow solution was kept undisturbed at 30 °C for at least 1 h. The gold seeds were generated. For the growth of the GNRs,

the growth solution was prepared by mixing CTAB (10 mL, 100 mM), AgNO₃ (62.5 µL, 10 mM), HAuCl₄ (425 µL, 10 mM) and ascorbic acid (67.5 µL, 100 mM). After mixing, the seed solution (50 µL) was added to the growth solution and the outcome solution was kept undisturbed at 30 °C for at least 12 h. The obtained GNRs were collected and purified by centrifugation (two times at 10,000 rpm, 10 min each) and washed by deionized water for two times to remove the excess CTAB.

2.3 Synthesis and modification of SBA-15

2.3.1 Synthesis of mesoporous silica SBA-15: SBA-15 was synthesized according to a published protocol [56]. Briefly, 4 g of EO₂₀PO₇₀EO₂₀ (Pluronic P123) was dissolved in the solution of 30 g of distilled water and 120 g of 2 M HCl at 35 °C. Then, 8.5 g of tetraethylorthosilicate (TEOS) was added dropwise while stirring. Mixing was continued for 10 h at 35 °C then 48 h at 60 °C. The mixture was then transferred into a teflon-lined autoclave and kept at 100–120 °C for another 48 h under static conditions. Finally, the solid product was recovered, washed with deionized water, and air-dried.

2.3.2 Synthesis of mesoporous thiol-functionalized SBA-15: Thiol-functionalized SBA-15 was synthesized by a method by Sarkar et al. [57]. Briefly, a solution of (3-mercaptopropyl)trimethoxysilane (MPTMS) (94 mg, 0.48 mmol) in 12 mL dry toluene was added to mesoporous silica SBA-15 (1.0 g) and the mixture was under continuous stirring at 105 °C for 12 h. After completion of the reaction, the reaction mixture was allowed to cool to room temperature, and the mesoporous powder was filtered, washed with CH₂Cl₂, and dried at room temperature in air.

2.4 Synthesis of GNRs@SBA-15-SH

To functionalize the GNRs with thiol groups on SBA-15, to 5 mL of GNRs solution, 156 µL of SBA-15-SH (1% wt.) dispersed into deionized water was added. After stirring overnight at room temperature, GNRs@SBA-15-SH were obtained by centrifugation (two times at 11,000 rpm, 10 min each) to remove the excess SBA-15-SH and then the SBA-15-conjugated GNRs were resuspended in the 5 mL of deionized water.

2.5 Characterization of GNRs

The nanoparticles' morphology was characterized by transmission electron microscopy (TEM) (LEO-906E-80 KV) images. The images were analyzed to determine the average diameter, length and size distribution of GNRs using ImageJ software. Ultraviolet (UV)–vis–NIR absorption spectra were recorded using a TU-1901 spectrometer. Fourier transform infrared (FTIR) spectra were recorded using BOMEM MB-Series 1998 FT-IR spectrometer. Zeta-potential of the nanoparticles were performed using a Malvern Zeta sizer Nano ZS instrument (Malvern Instruments, U.K.). A continuous wave laser (808 nm, 7 mm, 1.4 W, 3.6 W/cm²) was applied by a GCSLS-05–7W00 fiber-coupled diode laser system (Daheng Science&Technology, China). The amount of Au in samples was determined using an ICP-OES (Optima 8300, Perkin Elmer). Energy-dispersive X-ray spectroscopy (EDX) and EDX

elemental mapping analysis were performed by FEI Tecnai G2 F20 S-TWIN at 200 kV.

The concentration of Au atoms in the GNRs was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) following digestion. Briefly, 1 mL of the GNR solution was digested by addition of 2 mL of aqueous mixture of nitric acid (65%)/hydrochloric acid (37%) solution (1:3 ratio) at 50 °C. Then the sample was cooled to room temperature and diluted with deionized water up to 10 mL, followed by inductively coupled plasma - optical emission spectrometry (ICP-OES) analysis.

2.6 Evaluation of the photothermal heating capacity of GNRs@SBA-15-SH

To measure the photothermal capacity of the nanocomposites, 100 µL of GNRs@SBA-15-SH solutions with different concentrations were added to 96-well plates with control wells. Then all the solutions were irradiated by 808 nm diode laser with a 7 mm beam diameter for 2–10 min at 3.6 W/cm². The temperatures of the solutions were determined by directly placing a thermocouple into the solutions.

2.7 Drug loading and release study

2.7.1 Drug loading: For drug loading, DOX (1 mL, 5 µM) was added to the GNRs@SBA-15-SH (5 mL, 228 nM) in PBS buffer. After stirring overnight at room temperature, DOX-loaded GNRs@SBA-15-SH nanocomposite was obtained by centrifugation (two times at 11,000 rpm, 10 min each) to remove the excess free drug. DOX loading was determined indirectly by measuring the concentration of free DOX in the supernatant following centrifugation using UV–Vis spectrophotometer at 485 nm. The percentage drug loading efficiency of DOX was measured using the Equation (1):

$$\text{DLE \%} = \frac{\text{Abs}(\text{original DOX}) - \text{Abs}(\text{residual DOX})}{\text{Abs}(\text{original DOX})} \times 100\% \quad (1)$$

2.7.2 In vitro drug release of DOX: The release of drug was studied from GNRs@SBA-15-SH/DOX (5 mL, with DOX equivalent concentration 5 µM) in phosphate buffered saline (PBS) at pH (7.4 or 5.5) with and without NIR laser irradiation. Irradiated samples were exposed to NIR laser for 10 min at the intensity of 3.6 W/cm² and then the test tubes of all samples (with and without NIR laser irradiation) were shaken at 130 rpm in the dark. Finally, the samples were centrifuged at different time intervals and then the amount of released DOX in the supernatant was measured by UV–Vis spectroscopy at 485 nm.

2.8 Cell culture

Human lung carcinoma (A549) cells were used to investigate the biocompatibility of GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX as well as the chemo/photothermal synergistic therapeutic effect versus control individual treatments. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 10% v/v fetal bovine serum (FBS) and 1% antimycotic solution in a humidified atmosphere at 37 °C with 5% CO₂.

2.9 Cellular uptake

A549 cells were seeded in a six-well plate at a density of 100,000 cells per well and incubated for 24 h to reach confluency. Before the experiment, the culture medium was removed and 1 mL of GNRs@SBA-15-SH/DOX nanoparticles at a series of concentrations (0.78, 1.56, and 3.125 nM) dispersed in complete culture medium with supplements were added. The cells were incubated at 37 °C for 48 h. After that, the cells in each well were washed with PBS two times, trypsinized, and counted. After centrifugation at 5000 g for 5 min, the acquired cell pellets were dispersed in sodium hydroxide (10 M) to dissolve the silica coating. The suspended nanoparticles were digested using aqueous mixture of nitric acid (65%)/hydrochloric acid (37%) solution (1:3 ratio). The gold content of the resultant solution was assessed via ICP-OES. Finally, the percentage of cell uptake of gold atoms per cell was calculated using Equation (2) where C_e is concentration of gold atoms in the cells, C_i is concentration gold atoms in the incubated dose, N_e is number of cells 48 h after treatment and N_i is number of cells seeded into each well:

$$\text{Cell uptake by per cell (\%)} = \frac{C_e}{C_i} \times \frac{N_i}{N_e} \times 100 \quad (2)$$

2.10 Cell viability assay

Viability of A549 cells following exposure to free DOX, GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX against lung cancer A549 cells was determined using the MTT assay. Cells were seeded into a 96-well plate (5×10^3 cells/well) in 100 μ L DMEM (with 1% v/v FBS) for 24 h to reach confluency. Then the culture medium was removed and replaced with medium containing various concentration of free DOX (0–100 μ M) and GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX with the equivalent gold nanorods concentrations (0–12.5 nM). After incubation for 48 h, the supernatant was removed and 30 μ L of MTT solution (5 mg/mL in phosphate buffer solution) was added to each well for approximately 4 h in the dark. Then 120 μ L of DMSO was added to dissolve the dye, and absorption at 490 nm was measured by using a microplate reader (Bio-Tekn Elx 800).

For the NIR irradiation experiments, cells were cultured following the same protocol. Cells were incubated with GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX (with the equivalent GNRs concentrations) at different concentrations at 37 °C for 42 h before being irradiated by a NIR laser with a wavelength of 808 nm (3.6 W/cm² for 10 min). This was followed by further 2 h incubation. Finally, cell viability was evaluated using MTT assay (Equation (3)).

$$\text{Cell viability (\%)} = \frac{(\text{Mean Abs. of treatment group})}{(\text{Mean Abs. of control group})} \times 100 \quad (3)$$

2.11 Statistical analysis

Statistical analysis of the cell viabilities under different concentrations and different treatments using GraphPad Prims v.8.4 were compared by *t*-test, one- and two-way analysis of variance (ANOVA). A probability *p*-value < 0.05 was considered statistically significant. Experiments were carried out in triplicate and repeated three times independently.

3 Results and discussion

3.1 Characterization of GNRs@SBA-15-SH/DOX nanocomposite

The GNRs@SBA-15-SH/DOX nanocomposite was synthesized according to the protocol depicted in Figure 1. First, GNRs were prepared using the seed-mediated method and the TEM images of the obtained CTAB-coated GNRs demonstrated the average width and length of 11 ± 0.5 and 34 ± 2 nm, respectively (about 3:1 aspect ratio) as analyzed by ImageJ software (Figure 2(a)). Statistical analysis of the length and the diameter of more than 100 AuNRs is shown in Figure 2(b) and (c). ICP-OES was used to determine the concentration of Au atoms in the gold nanorods (Table S1). In the following step, SBA-15 nanorods with high surface area were synthesized and functionalized by post grafting method using 3-mercaptopropyl trimethoxysilane (Figure 1). The successful incorporation of thiol onto the SBA-15 nanorods was verified by the FTIR spectra.

The FTIR spectra of SBA-15 and SBA-15-SH are shown in Figure 3. As shown in Figure 3, SBA15-SH has a very weak vibrational band at 2570 cm⁻¹ assigning to the S-H stretching vibration, while this band is blank for SBA-15. This qualitative evidence from FTIR spectra indicates that the thiol groups were successfully incorporated into the SBA-15 nanorods [58].

SBA-15-SH nanorods were then used as a uniform mesopore with good water dispersibility for coating GNRs (Figure 1). Successfully prepared SBA-15-SH and GNRs@SBA-15-SH nanocomposite were imaged using TEM (Figure 4(a) and (b)). Finally, GNRs@SBA-15-SH nanocomposite was used as a nanocarrier for an anticancer drug (Doxorubicin hydrochloride). The TEM images of the GNRs@SBA-15-SH/DOX nanocomposite (Figure 4(c) and (d)) and (Figure S1) confirm the loading of DOX into the GNRs@SBA-15-SH nanocomposite.

Successful synthesis of GNRs, GNRs@SBA-15-SH, and GNRs@SBA-15-SH/DOX were also studied by UV-Vis absorption spectroscopy (Figure 5). CTAB-capped GNRs demonstrated a strong longitudinal SPR band at around 710 nm, which was red shifted by about 37 nm on coating by mesoporous silica SBA-15-SH, whereas the transverse plasmon band (516 nm) remained approximately unchanged. This phenomenon could be explained by the fact that local refractive index of mesoporous silica layer (1.45) is larger than water (1.33) [59]. After loading of DOX, an increased UV-Vis absorption peak revealed at around 757 nm.

N₂ adsorption-desorption isotherms of SBA-15, SBA-15-SH, and GNRs@SBA-15-SH nanocomposite were shown

Figure 1: Schematic illustration the synthesis of the GNRs@SBA-15-SH/DOX nanostructure.

in Figure 6(a). As shown all samples are typical type IV with an HI hysteresis loop, which is characteristic of materials with mesoporous characteristic. Moreover, the pore diameter of GNRs@SBA-15-SH is smaller than that of SBA-15 as show in the pore size distribution (Figure 6(b)), suggesting that GNRs is introduced into the pores rather than on the exterior of SBA-15-SH.

The spatial distribution and amount of loaded Au on GNRs@SBA-15-SH were determined by elemental mapping and energy-dispersive X-ray spectroscopy (EDX). As shown in Figure 7, the nanocomposite contains 88.9% of Au.

Ligand exchange was confirmed by surface electric potentials measurements of the GNRs, CTAB-coated GNR, GNRs@SBA-15-SH, and GNRs@SBA-15-SH/DOX (Figure 8(b)). DLS measurements (Figure 8(a)) showed that CTAB-capped GNRs had the hydrodynamic size of 54 nm and zeta potential of +39.2 mV. When the GNRs were modified with SBA-15-SH, the average hydrodynamic size increased to 59 nm and the zeta potential decreased to +27.3 mV. After loading of DOX into the GNRs@SBA-15-SH, the size of nanocomposite, GNRs@SBA-15-SH/DOX, was increased to 141 nm with a slight decrease in surface charges

(+26.3 mV). The expected increase in the size of nanoparticles after loading of DOX can probably be attributed to the effect of hydrogen bonding of hydroxyl and amine groups of DOX, with silanol hydroxyl groups of SBA which leads to aggregation.

These results suggest that SBA-15-SH to stabilize the adsorbed DOX. This enabled the formation of highly integrated DOX–GNR complexes in which DOX is entrapped in the hydrophobic interior of the SBA-15-SH monolayer.

The FTIR spectra of GNRs, GNRs@SBA-15-SH, GNRs@SBA-15-SH/DOX, and DOX were recorded in Figure 9. The FTIR spectrum of DOX molecules showed existence of the two peaks at 1622 and 1584 cm^{-1} (N–H), and one at 1725 cm^{-1} to the (C=O) of carbonyl group, that are rather weak in the FTIR spectrum of GNR@SBA-15-SH-DOX nanocomposite, indicating the formation very weak intermolecular of hydrogen bonds between DOX molecules with the silanol groups on the external surface of SBA-15-SH. The disappearance of the –SH peak in GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX indicates the formation of the coordination bonds between the –SH groups of SBA-15-SH and GNRs.

Figure 2: Characterization of the GNRs.

(a) TEM image of CTAB-capped GNRs, (b) and (c) Statistical data of the length and the diameter of more than 100 GNRs as shown in (a), respectively.

3.2 Photothermal capacity of GNRs@SBA-15-SH solutions

The photothermal capacity of GNRs@SBA-15-SH nanocomposite at different concentration were compared in Figure 10(a). The results showed that upon 808 nm NIR laser irradiation at a power density of 3.6 W/cm^2 , the temperature increased with increasing GNRs@SBA-15-SH concentrations in the dispersion. To examine the photothermal effects of GNRs@SBA-15-SH, dispersion of GNR and GNRs@SBA-15-SH with the same GNR concentration (12.5 nM) and solution of PBS and DOX were irradiated with a NIR laser (3.6 W/cm^2). The temperature of the

GNRs@SBA-15-SH and GNR suspension increased from 23 to 54°C and 23 to 62°C respectively within 10 min (Figure 10(b)), while the temperature of controls (PBS and DOX) showed no significant increase in temperature under the same conditions. The results demonstrated that GNRs@SBA-15-SH nanocomposite have great potential for efficient light-to-heat conversion.

3.3 DOX loading and release

Nanocomposites could exhibit high DOX loading up to $43 \text{ wt.}\%$. Drug release at pH 7.4 and 5.5 with or without

Figure 3: FTIR spectra of (a) SBA-15-SH and (b) SBA-15.

Figure 4: TEM images of (a) SBA-15-SH, (b) GNRs@SBA-15-SH, and (c), (d) GNRs@SBA-15-SH/DOX, respectively.

Figure 5: The UV-Vis absorption spectra of GNRs, GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX.

Figure 6: (a) N_2 adsorption-desorption isotherms and (b) pore size distributions of SBA-15, SBA-15-SH, and GNRs@SBA-15-SH nanocomposite.

NIR laser irradiation in PBS solutions was studied to simulate neutral environment of blood flow and acidic one in cellular endosomes, respectively (Figure 11). Both NIR light and pH were shown efficient to control the DOX

release from GNRs@SBA-15-SH/DOX nanocomposites. As shown in Figure 11, the extent of DOX released increased with time and the rate of DOX release from the pores of GNRs@SBA-15-SH/DOX was dependent on the pH of the

Figure 7: EDX and EDX maps of GNRs@SBA-15-SH nanocomposite, Au, O, Si and combined.

Figure 8: (a) Hydrodynamic size analysis by DLS and (b) Surface zeta potential of GNRs (CTAB stabilized), GNRs@SBA-15-SH, and GNRs@SBA-15-SH/DOX.

Figure 9: FTIR spectra of (a) GNRs, (b) GNRs@SBA-15-SH, (c) GNRs@SBA-15-SH/DOX, and (d) DOX.

medium. Without NIR laser irradiation, GNRs@SBA-15-SH/DOX nanocomposite showed higher release rate of DOX molecules at pH 5.5 compared to that at pH 7.4 [60].

Acidic conditions enhanced drug release via protonation of silanols resulting in dissociation of electrostatic bond between the negatively charged silanols groups of the nanocomposite and the positively charged DOX molecules [61, 62]. Under these conditions, drug release was also triggered by the high solubility of DOX at pH 5.5. An important barrier to DOX release at high pH is its negligible solubility at this pH. The pH-responsive controlled DOX release has the advantage of reducing burst release of the drug thus limiting off-target effects of DOX, as well as enhancing the antitumor efficacy by releasing the drug in the acidic intracellular environment of cancer cells [63]. The effect of NIR light on controlled drug release from GNRs@SBA-15-SH/DOX nanocomposite in PBS solutions at pH 7.4 and 5.5 by NIR laser at 808 nm was examined. DOX release from GNRs@SBA-15-SH/DOX was considerably fast in PBS at pH 5.5 under NIR laser irradiation (3.6 W/cm² power density for 10 min), with % cumulative DOX release reaching 97% at 24 h and was almost 3 times higher than that at pH 5.5 without NIR irradiation. Furthermore, GNRs@SBA-15-SH/DOX nanocomposites at pH 7.4 were also irradiated by NIR laser at 808 nm. Similarly, after 24 h, the cumulative release of DOX reached following irradiation to NIR (55%) was higher than in absence of NIR radiation (17%). The results indicated that NIR is converted into heat energy which dissociates the weak electrostatic bond between DOX and the carrier.

Therefore, the GNRs@SBA-15-SH/DOX nanocomposite demonstrated pH and NIR laser dual-stimulus responsive

drug release performance, improving the efficiency of targeted drug delivery.

3.4 Cellular uptake

ICP-OES was used to determine the concentration of Au atoms in the cells exposed to the nanocomposites. The quantitative analysis of the cellular uptake by ICP-OES showed that the concentration of Au atoms in the cells after 48 h incubation with different concentrations of GNRs@SBA-15-SH/DOX (0.78, 1.56, and 3.125 nM or 0.316, 0.632, and 1.265 ppm) was 0.115, 0.185, and 0.556 ppm, respectively, and the uptake efficiency was determined by the percentage of gold concentration to incubated dose per cell using the Equation (2).

To determine that nanoparticles are internalized or just attached to the cell surface, the cells were thoroughly washed with PBS before the ICP. Therefore, ICP analysis shows the concentration of nanoparticles that have penetrated into the cells. In addition, the washing solution was tested by ICP and the nanoparticles were not approved. The internalized amount of gold atoms per cell was 0.37, 3.66, and 5.5% after 48 h incubation, respectively, which confirms that cell uptake of GNRs@SBA-15-SH/DOX.

According to previous studies, the cellular uptake mechanism of mesoporous silica nanoparticles is through endocytosis. When the nanoparticles approaches the cells, the cell membrane invagination begins, then the phagocytosis process of nanoparticles which leads to formation of intracellular endosomes happens. It is a well-known fact that the intracellular location of DOX is within the nucleus.

Figure 10: (a) Temperature elevation of the solutions containing different concentration GNRs@SBA-15-SH (0, 1.56, 3.125, 6.25, 12.5, and 25 nM) after 808 nm laser irradiation (3.6 W cm^{-2}); (b) Temperature elevation of PBS, DOX, GNRs, and GNRs@SBA-15-SH (equivalent GNR concentration: 12.5 nM) after irradiating with different 808 nm laser intensities (3.6 W cm^{-2}).

Ma et al. reported the red purple signals of nucleus in confocal microscopy images of DAPI staining which was the mixture of the red and blue that comes from the DOX and DAPI staining that made the nucleus blue, respectively [64].

3.5 In vitro cell viability assay

Cell viability of human lung cancer cells (A549 cells) following exposure to free DOX, GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX were assessed by MTT assay. First, for the control experiment (not loaded with DOX and without NIR irradiation), we evaluated the biocompatibility of GNRs@SBA-15-SH nanocomposite *in vitro*. The cells were incubated with different concentrations of

GNRs@SBA-15-SH (ranging from 0.39 to 12.5 nM) for 48 h (Figure 12(a)) and the cell viability was higher than 80% after incubation. The results exposed that no cytotoxicity was observed and the nanocomposite has good biocompatibility. The good biological safety of GNRs@SBA-15-SH nanocomposite is a prerequisite for their use in the biomedical area. The loading of the drug to nanocomposite led to reduced IC₅₀ (the concentrations that inhibited cell growth by 50%) and thus a better cell killing effect of the drug. The GNRs@SBA-15-SH/DOX showed lower IC₅₀ than free DOX in A549 cells. Figure 12(a) and (b) showed the cell viability of A549 cells treated with different concentrations of free DOX and DOX loaded composites. The cell viability values were 43 and 53%, respectively. As shown in Figure 12(a), statistically significant difference in cell

Figure 11: Cumulative DOX release from GNRs@SBA-15-SH/DOX nanocomposite with and without NIR laser irradiation at different pH values.

viability was observed by *t*-test between treated cells with 6.25 nM ($p = 0.0037$) and 12.5 nM ($p = 0.004$) concentrations of GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX than treated cells with 0.39, 0.78, 1.56, and 3.125 nM concentrations. Also in Figure 12(b) the results were compared to the control group and analyzed by one-way ANOVA ($p < 0.0001$). Table 1 shows IC₅₀ values of free DOX, GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX (GNRs equivalent concentrations) against A549 cells.

Next, to evaluate the chemo-photothermal therapeutic efficiency of GNRs@SBA-15-SH/DOX, we compared the cytotoxic effects of either of GNRs@SBA-15-SH (PTT) and GNRs@SBA-15-SH/DOX (combined PTT and chemotherapy) without and with laser irradiation in A549 cells using MTT assay (Figure 13(a)). The cells were incubated with GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX at GNRs equivalent concentrations (0.78, 1.56, and 3.125 nM) for 42 h. Then after NIR laser irradiation for 2–10 min, the cell medium was heated to about 37, 42 and 47 °C, we examined the therapeutic efficiency of GNRs@SBA-15-SH-NIR and GNRs@SBA-15-SH/DOX-NIR after incubation for 4 h by MTT assay. As shown in Figure 13(a), cell viabilities were determined as a function of incubation concentration of nanocomposites at three dosages and results were analyzed by two-way ANOVA. On NIR laser irradiation for 10 min, increasing the concentration of GNRs@SBA-15-SH was accompanied with a reduction in cell viability and results showed significant changes in cell viability at a concentration of 3.125 nM ($*p = 0.03$) but no statistically significant difference was observed at concentrations of 0.78 and 1.56 nM ($p = 0.9$ and 0.6). Similarly, when

A549 cells were irradiated by NIR laser for 10 min (3.6 W cm^{-2}) after incubation with GNRs@SBA-15-SH/DOX at GNRs equivalent concentrations (0.78, 1.56, and 3.125 nM), significant reduction in cell viability was compared to GNR@SBA-15-SH/DOX without laser irradiation and results showed significant changes in cell viability at concentration of 3.125 nM ($p = 0.03$) but no statistically significant difference was observed at concentrations of 0.78 and 1.56 nM ($p = 0.99$ and 0.35). Notably, the viability of cells treated with 3.125 nM GNRs@SBA-15-SH nanocomposite decreased from 91% to about 53% on exposure to NIR radiation, confirming the photothermal effect on cells. The synergistic effect of PTT and chemotherapy using the GNRs@SBA-15-SH/DOX was elucidated from the significantly higher efficiency for killing A549 cancer cells (cell viability = 27%) compared to all other experiments *in vitro* at a concentration of 3.125 nM GNRs@SBA-15-SH/DOX (the amount of DOX was 0.078 μM) following exposure to NIR.

We suggest that thermal effect of the PTT enhanced cell uptake into A549 cells, and stimulated drug release. This could justify the synergistic effect between the chemotherapy and PTT. To assess existence a synergist effect in the combination treatment in this system, we compared the cell viability to the computed value from the additive effect. In particular, we examined the cell viabilities of GNRs@SBA-15-SH/DOX without laser irradiation, GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX under laser irradiation MTT assay (Figure 13(b)) and results were analyzed by two-way ANOVA. These three different types have an equivalent concentration of Au nanorods (3.125 nM) and the DOX

Figure 12: (a) Cell viabilities of the A549 cells after incubation with various concentrations of GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX, at concentration of 6.25 nM $^{**}p = 0.0037$ and at concentration of 12.5 nM $^{**}p = 0.004$ (b) free DOX, $^{****}p < 0.0001$, $n = 3$.

Table 1: IC₅₀ of free DOX, GNRs@SBA-15-SH, and GNRs@SBA-15-SH/DOX after 48 h incubation in A549 cells.

Free DOX	GNRs@SBA-15-SH	GNRs@SBA-15-SH/DOX
1.302 μM	5.448 nM	3.304 nM

concentration 0.078 μM. When A549 cells were incubated with GNRs@SBA-15-SH/DOX for 48 h, cell viability due to the chemotherapy was about 65%. Next, we incubated A549 cells with GNRs@SBA-15-SH and GNRs@SBA-15-SH/DOX for 42 h, followed by laser exposure and the cell medium was heated to about 37, 42 and 47 °C. Here we exert the additive cell viability (f_{additive}) that was calculated from a relation of $f_{\text{additive}} = f_{\text{chemotherapy}} \times f_{\text{PTT}}$ where f is the cell viability of each treatment. When the fraction of cell viability from the combination treatment ($f_{\text{combination}}$) is lower than

f_{additive} , there is a synergistic effect. When $f_{\text{combination}} = f_{\text{additive}}$, it is an additive effect [65]. The experimental cell viability from the combination treatment ($f_{\text{combination}}$) value of GNRs@SBA-15-SH/DOX+Laser at 42 and 47 °C was 31 and 21%, which was lower than the calculated cell viability by additive interaction of PTT and chemotherapy (f_{additive}) value (34 and 32%) although showed no significant changes in cell viability ($p = 0.98, 0.6$). This is indicative of the synergistic anticancer therapeutic effect of GNRs@SBA-15-SH/DOX-NIR. Figure 13(b) also shows the enhanced therapeutic efficacy by GNRs@SBA-15-SH/DOX-NIR at a temperature of 42 and 47 versus 37 °C. Chemo-photothermal therapy of DOX loaded nanocomposite illustrated a synergistic effect.

Our results demonstrate that compared with individual PTT/chemotherapy, combined chemo-photothermal therapy showed superior efficiency. Four feasible reasons

Figure 13: (a) Relative viabilities of A549 cells incubated with GNRs@SBA-15-SH or GNRs@SBA-15-SH/DOX at different concentrations under exposure to a 808 nm laser at a power density of 3.6 W/cm^2 for 10 min, $*p < 0.05$, $***p = 0.0002$, $n = 3$, (b) A comparison of cell viability for A549 cells treated by GNRs@SBA-15-SH+Laser, GNRs@SBA-15-SH/DOX and GNRs@SBA-15-SH/DOX+Laser. For photothermal treatment, the media were under 808 nm laser irradiation for 10 min, corresponding to the maximum temperature increases to 37, 42, and 47 °C. The additive therapeutic efficacies of combined treatment at each temperature were calculated by using the relation of $f_{\text{additive}} = f_{\text{chemotherapy}} \times f_{\text{PTT}} \times 100$, here f is the cell viability of each treatment, $*p = 0.0185$, $**p = 0.0021$, $n = 3$.

can explain this synergistic effect: (1) Heat created via GNRs resulting from NIR laser irradiation could damage A549 cancer cells directly [66, 67]; (2) the fast release of DOX from GNRs@SBA-15-SH/DOX nanocomposite was triggered by NIR laser irradiation and cause subsequent cell damage; (3) It is conceivable that the cytotoxicity of DOX increased by heating [68, 69]; and (4) synchronous performance of PTT and chemotherapy was synergistic for killing A549 cancer cells.

Considering all aspects, we believe that among the functionalized GNRs with stimulus-responsive polymers, porous silica, DNA, proteins, and porous metal–organic framework, the GNRs coated with Thiol-functionalized SBA-15 (GNR@SBA-15-SH) indicated excellent potential for fulfilling the idea of combined chemo-photothermal therapy. In addition this nanocomposite exhibits high loading DOX capacities.

4 Conclusion

In conclusion, GNRs@SBA-15-SH/DOX nanocomposite was successfully constructed as a synergistic therapy device for delivery heat and drug to cancer cells. These novel nanocomposites demonstrated high photothermal conversion efficiency and excellent biocompatibility *in vitro* and also efficient carrier for DOX with high loading capacity and pH-responsive release due to the electrostatic interactions between silanol groups on the external surface of SBA-15-SH and DOX molecules for chemotherapy of cancer cells. In addition, we indicated when GNRs@SBA-15-SH/DOX nanocomposite was irradiated by 808 nm laser, both photothermal-/chemo- therapy were at the same time driven, resulting in significant enhancement of therapy effects for killing A549 lung cancer cells than the monotherapies

in vitro. Therefore, GNRs@SBA-15-SH/DOX nanocomposite by the combination of photothermal therapy and chemotherapy has great potential in cancer therapy. In clinical practice, these nanocomposites are intended for local injection in the tumor tissue, or for systemic application following surface functionalization with specific ligands targeting the tumor cells.

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