A theranostic plaster combining photothermal therapy and photodynamic therapy based on chlorin e6/gold nanorods (Ce6/Au nrs) composite

Jing Yan, Haifeng Sun, Jingchen Li, Wei Qi*, Hua Wang*

Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu, Shandong 273165, China

GRAPHICAL ABSTRACT

ABSTRACT

In this work, a new type of plaster based on chlorin e6 (Ce6)-gold nanorods (Au nrs) complex was developed for near-infrared (NIR) cancer therapy. The cancer treatment could be performed by applying the plaster to the tumor site and using dual-wavelength laser irradiation, 660 nm and 808 nm. The plaster could be irradiated repeatedly during the treatment and was completely noninvasive. It has exhibited broad, strong absorption in the NIR region, excellent photothermal efficiency and high singlet oxygen generation. The prepared plaster had low dark toxicity, while under irradiation, it showed high cell killing ability using human squamous cell carcinoma cells. The combinational effect of photothermal therapy (PTT) and photodynamic therapy (PDT) was also demonstrated in vivo. The plaster-treated tumor bearing mice showed highest therapeutic efficiency, compared to that treated with single PTT or PDT. The tumors have been destroyed completely after 16 days treatment with the double phototherapy treatment. Therefore, the developed system might be potential as a dual-modal phototherapy way for cancer treatment in clinical application.

1. Introduction

Since cancer has become one of the greatest threats to human health, the development of therapeutic strategies with high specificity and efficiency is highly desired. In recent years, phototherapies including photothermal therapy (PTT) and photodynamic therapy (PDT) have attracted tremendous attention due to their minimal invasiveness, remote controllability, and precise spatial-temporal selectivity [1,2]. PTT involves employing photo-absorbing agents to convert light energy into heat, which can cause irreversible cellular damage and subsequent tumor destruction [3]. A variety of different photothermal transducers have been reported including organic compounds [4–6], metal nanostructures [7–10] and carbon-based materials [11–14]. Gold nanostructures are the most attractive photo-absorbing agents because their localized surface plasmon resonance (SPR) endows them with the capability to absorb light at a specific wavelength, and the SPR peaks can be easily tuned by altering their size, shape, structure or a combination of these parameters [15]. For instance, the excitation

* Corresponding author.
E-mail addresses: qf_qw@163.com (W. Qi), huawangqfnu@126.com (H. Wang).

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wavelength for gold nanorods (Au nrs) is in near-infrared (NIR) region. The use of NIR light is preferred for the thermal therapy in that water, melanin and hemoglobin have absorption minima between 700 and 900 nm, and the light at this wavelength is most likely to pass directly through tissues without significant heat generation [16,17]. PDT uses photosensitizers (PSs) to kill tumor cells under light exposure by generating reactive oxygen species (ROS) such as singlet oxygen \( (^1O_2) \) [18]. Chlorins have been used frequently as PS agents because of their high extinction coefficient in red light region and high singlet oxygen quantum yield, among which, chlorin e6 (Ce6) is the most commonly used in that it can be activated by NIR light and eliminated rapidly from the body [19,20].

Despite their potential in cancer treatment, there are still some challenges for phototherapies. For example, PDT treatment is often repeated because of tumor recurrence resulting from the easy clearance of photothermal nanotherapeutics from the tumors [21]. While in the case of PDT, its application in practical clinic has been limited by their relatively low efficiency due to the hydrophobicity and easy aggregation of PSs in aqueous solution [22]. To solve these problems, there are great needs to combine PTT and PDT to treat cancers. In fact, it has been shown that combined therapies exhibited higher therapeutic efficiency compared to any single treatment because each modality has its own advantages and limitations [23–26].

In this study, we developed a nanocomposite plaster composed of PEI-Ce6 and PSS-Au nrs combining PTT and PDT. Ce6 grafted to polyethyleneimine (PEI) functioned as a PDT agent and Au nrs modified with poly(styrene sulfonate) (PSS) were used as PDT agents. Here, polyelectrolyte PEI and PSS were proposed to improve the water-solubility of Ce6 and the dispersion behavior of Au nrs, respectively, as well as to facilitate the following assembly. The plaster was prepared using nonwoven fabric as substrate. With the plaster, we studied its synergistic PTT and PDT effects in vitro using A431 cell line (human squamous cell carcinoma cells) and in vivo with the squamous cell carcinoma-bearing mice. It was demonstrated that the combinational therapy of the plaster was much more efficient in treating the skin cancer than single treatment of PTT or PDT. Moreover, there are two important advantages in this kind of polymer/nanomaterials composite plaster: (1) it could repeatedly treat tumors with total noninvasiveness; (2) it would be highly efficient in treating superficial tumors. Taken together, this work proposed a strategy to fabricate a noninvasive and efficient plaster for combinational therapy of superficial tumors.

### 2. Materials and methods

#### 2.1. Materials

Polyethyleneimine solution (PEI, \( \sim 50\% \) in H₂O, Mw: \( \sim 25,000 \), poly(styrene sulfonate) (PSS, Mw: \( \sim 70,000 \), doxorubicin hydrochloride and gold(III) chloride trihydrate (HAuCl₄·3H₂O), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC hydrochloride) and N-Hydroxysuccinimide (NHS) was purchased from Sigma-Aldrich (USA). Expandable graphite (EG) 8099200 was purchased from Qingdao BCSM Co., Ltd. (China). Cetyltrimethylammonium bromide (CTAB), NaBH₄, AgNO₃, HCl, NaOH was gained from Tianjin Damao Chemical Reagent co., Ltd. (China). Chlorin e6 (Ce6) was purchased from J & K Scientific Ltd. Dimethyl sulfoxide (DMSO), 98% H₂SO₄, 30% H₂O₂, and KMnO₄ were purchased from Shanghai Chenyun Chemical and Engineering Company. All the chemical reagents were used as received without further purification. The water used in this work has been purified with Millipore Milli-Q system (USA).

#### 2.2. Preparation and surface modification of gold nanorods

Au nrs were synthesized by traditional seed-mediated method [27]. Firstly, gold seeds were prepared by mixing 250 µl 0.1 M HAuCl₄ and 9.75 ml 0.1 M CTAB, followed by the addition of 600 µl 0.01 M ice-cold NaBH₄. The above system was cultured for 2 h at 28–30 °C. Then nanorods growth solution was prepared by mixing 0.01 M HAuCl₄ (2 ml), 0.01 M AgNO₃ (400 µl), 0.1 M HCl (800 µl) and 0.1 M CTAB (40 ml). When the solution became colorless, 200 µl gold seed solution was added and the system stayed at 30 °C overnight. Finally, the Au nrs were collected and purified by centrifugation (10000 rpm, 10 min). The centrifugated Au nrs were suspended in ultrapure water for use.

Au nrs surface modification was performed with PSS [28]. Briefly, 5 ml 2 mg/ml PSS (in 6 mM NaCl) was injected into 5 ml Au nrs dispersion with gently stirring. Then, the mixed system was continuously stirred for another 3 h. At last, the resultant PSS-Au nrs were collected to remove excess PSS by centrifugation at 10,000 rpm/min, 10 min and re-suspended in ultrapure water.

#### 2.3. Preparation of PEI-Ce6 conjugates

As reported [29], 10 mg Ce6 was dissolved in 500 µl DMSO and kept stirring until it completely dissolved, then EDC (20 mg) and NHS (20 mg) were quickly added and kept activated for 4.5 h. Subsequently, 1 mg/ml PEI solution (1 ml) was injected into the above mixture. The mixed system was stirred for another 12 h at room temperature in dark. The excess Ce6 and other chemicals were removed by ultrafiltration. Then product was redispersed in fresh pure water and stored at 4 °C for use.

#### 2.4. Preparation of PEI-Ce6/PSS-Au nrs multilayer

Glass slide, silicon wafer, quartz slide and nonwoven fabric were used as substrate for different characterization. The glass slide, silicon wafer and quartz slide were treated before use with piranha solution (98% H₂SO₄:30% H₂O₂ = 7:3) overnight at room temperature, washed with deionized water and finally dried under nitrogen. For the nonwoven fabric, to be more hydrophilic, it was treated with plasma cleaner for 5 min before assembly. PEI-Ce6/PSS-Au nrs were constructed by alternate deposition of PEI-Ce6 and PSS-Au nrs onto the substrate. Each deposition was performed with the help of vacuum drying.

#### 2.5. Measurement of photothermal conversion efficiency

The photothermal effect induced by PEI-Ce6/PSS-Au nrs was investigated by irradiating the system with laser 808 nm (1.0 Wcm⁻²) and with 808 nm (1.0 Wcm⁻²) and 660 nm (0.5 Wcm⁻²) lasers, respectively. The temperatures were recorded every 2 min until 10 min. The same measurement was performed on PSS-Au nrs and PEI-Ce6 as controls. Temperatures for all the test systems were monitored using DAE-905k thermometer (SENDAE, China).

#### 2.6. Detection of reactive oxygen produced by PEI-Ce6/PSS-Au nrs

It is difficult to determine the ROS directly due to their short life time in solution. Usually, a chemical oxidation method by means of 9,10-anthracenediylbis (methylen) dimalonic acid (ABDA), a kind of trapping agent for single oxygen, was employed to monitor the capability of photosensitizers to generate \(^1O_2\). The reaction was monitored using UV–vis spectrophotometer by recording the decrease of the absorbance at 400 nm which is one of the characteristic absorption peaks
of ABDA [30,31]. In this experiment, the as-prepared PEI-Ce6/PSS-Au nrs was immersed in 1.0 ml water and was immediately irradiated upon adding ABDA/DMSO solution (10 mM, 25 μl) simultaneously by a 660 nm laser with a power intensity of 0.5 W cm⁻² for 0, 4, 8, 12, 16, and 20 min, respectively. The same experiments were performed using 808 nm (1.0 W cm⁻²) and 660 nm (0.5 W cm⁻²) simultaneously. The absorbance spectra of ABDA were all recorded at these time points. As a contrast, the production of ROS in Ce6 and PEI-Ce6 systems were monitored in the same way.

2.7. Characterization

In this study, all UV–vis absorption spectra were obtained with UV3600 spectroscopy (Shimadzu, Japan), and Fourier transform infrared spectroscopy (FTIR) spectra were performed on Tensor-27 (Bruker, Germany). The size and distribution of Au nrs was evaluated by transmission electron microscope (TEM) JEM-1011 (JEOL, Japan). The zeta-potentials of Ce6, PEI-Ce6, Au nrs and PSS-Au nrs were determined by Zetasizer Nano ZS ZEN 3600 (Malvern, UK). The surface morphology of PEI-Ce6/PSS-Au nrs was observed using Nanoscope IIIa apparatus (Digital Instruments, USA).

2.8. Cell culture

Human squamous cell carcinoma cells A-431 were purchased from Center for Type Culture Collection (CTCC) of Wuhan University. A-431 cells were cultured in Eagle’s minimum essential medium (EMEM) containing 10% (v/v) fetal bovine serum, 1.0 mM pyruvate sodium and 100 units/ml penicillin/streptomycin at 37 °C with 5% CO₂. Multiplication of cell in logarithmic phase was conducted with 0.05% trypsin.

2.9. Cytotoxicity

CCK-8 assay was carried out to evaluate the potential cytotoxicity of PEI-Ce6/PSS-Au nrs system. Briefly, cells (15,000 cells/ml) were seeded on the surface of PEI-Ce6/PSS-Au nrs, which has been placed in 24-well cell culture plates beforehand. Beside this group (PTT + PDT group), there are control groups including blank tissue culture polystyrene (TCPS) and blank glass slides, PSS-Au nrs group (PTT group), PEI-Ce6 group (PDT group). In each group, half of them were exposed to laser irradiation, including 808 nm (1.0 W cm⁻², 10 min), or 660 nm (0.5 W cm⁻², 10 min), or both. After irradiation, cells were kept being cultured for another 4 h, followed by CCK-8 assay procedure. Simultaneously, the live/dead cell double staining kit (Sigam-Aldrich, USA) is utilized for fluorescence staining of viable and dead cells. This kit contained calcein-AM (Ca AM) and propidium iodide (PI) solutions, which could stain viable and dead cells, respectively [32]. The fluorescence images were observed and recorded with confocal laser scanning microscopy (CLSM, Olympus FV 1000, Japan).

2.10. In vivo combinational PTT/PDT

In vivo animal experiments followed the guidelines and animal moral ethical responsibility. 4–6 weeks nude mice, supplied by Institute of Process Engineering, Chinese Academy of Sciences (Beijing, China), were used for comparing combinational therapy efficiency. A-431 cells (1.0 × 10⁷) in EMEM medium were injected into the right leg of mice. Tumor-bearing mice were divided into five groups, including one control group without any treatment, and another control group using blank nonwoven substrate, the PTT group using PSS-Au nrs, the PDT group using PEI-Ce6 and the PDT + PTT group using PEI-Ce6/PSS-Au nrs. In each group, the mice were further divided into two parts, one without laser irradiation and the other with laser irradiation. When the tumor volume grew to 70–90 mm³, the plasters were pasted onto the tumor and irradiated by 808 nm (1.0 W cm⁻², 10 min), or 660 nm (0.5 W cm⁻², 10 min), or both, accordingly. After laser irradiation, tumor volume of each group was measured with digital Vernier caliper every day during the following 16 days, and was calculated with the formula: tumor volume = 1/2 (length × width²) [33]. Moreover, the weight of mice of each group was monitored and recorded every day during the treatment. Simultaneously, the mice treated with PEI-Ce6/PSS-Au nrs were taken photos to show some observable changes of the tumor sites.

3. Results and discussion

Ce6 is a second-generation PS with antitumor activity when being irradiated by a specific laser. However, it has low water-solubility and aggregates easily in aqueous solution, which has limited its application in practical clinics. To overcome this barrier, Ce6 was grafted here to branched PEI, a hydrophilic polymer with abundant amino groups. An amido bond formed between them via a reaction between the amino group of PEI and the carboxyl group of Ce6 with EDC and NHS as coupling agents. The formation of PEI-Ce6 was confirmed by UV spectra, and FTIR spectra. From the UV absorption spectra (Fig. 1A), PEI-Ce6 showed the same characteristic absorption peaks as free Ce6 at 403 nm, 503 nm and 663 nm, respectively. Additionally, in the FTIR spectra of PEI-Ce6 (Fig. 1B), the absorption at 1650 cm⁻¹ became stronger comparing to that of Ce6 due to the formation of amido bond. Simultaneously, the absorption peaks at 2858 cm⁻¹, 2920 cm⁻¹ became stronger too, which could be attributed to the CH₂ stretching vibration of the PEI in PEI-Ce6. Furthermore, zeta-potentials of Ce6 and PEI-Ce6 were determined and listed in Table 1. Obviously, the potential shifted from −5.50 ± 1.1 mV to 32.2 ± 4.2 mV, which could be attributed to the strong positive charges of PEI macromolecules. The strong positive charge of PEI-Ce6 would effectively prevent the aggregation of it, which is helpful to fully show its photodynamic therapeutic effects in the following cancer treatment. Overall, all these results indicated the successful linkage between PEI and Ce6.

The Au nrs were fabricated with seeded growth method using CTAB surfactant. To make full use of the optical characteristics of Au nrs, surface modifications are usually important and essential. Here we utilized a polyion, PSS, to make the modification. Firstly, to evidence the modification, the surface charge of Au nrs and PSS-Au nrs in water were determined by using zeta potential technique. It was clear that the modification of Au nrs with PSS induced a charge reversal on the nanorods surface as zeta potential shifted from 43.4 ± 4.5 mV to −51.4 ± 5.4 mV (Table 1). Then, the formation of PSS-Au nrs was confirmed also using UV–vis spectroscopy. The absorption spectra of Au nrs in Fig. 2A showed two peaks, 510 nm and 850 nm. A small peak shift from 850 nm of Au nrs to 890 nm of PSS-Au nrs should be attributed to the change in the refractive index of the surrounding medium of Au nrs from air or CTAB to PSS [34]. Meanwhile, TEM images (Fig. 2B) showed the good dispersity and stability of the prepared PSS-Au nrs with a length of 70 nm length and a width of 17 nm.

The PEI-Ce6 and PSS-Au nrs were assembled alternately onto template substrates including quartz wafers, glass slides, or nonwoven fabrics, respectively. Using quartz wafers as substrates, UV–vis spectra of PEI-Ce6/PSS-Au nrs were obtained. From the UV–vis spectra of the composite PEI-Ce6/PSS-Au nrs (Fig. 3A), there were the characteristic absorptions of Ce6 at 403 nm, 503 nm, 663 nm and the SPR peak of Au nrs at 890 nm. And surface morphology of the composite PEI-Ce6/PSS-Au nrs characterized by AFM technique was shown in Fig. 3B, and one
could easily see the presence and distribution of Au nrs on the surface. For comparison, the morphology of the film only with PEI-Ce6 was also observed with AFM and shown in Fig. 3C, in which the height of surface was obviously lower than that of PEI-Ce6/PSS-Au nrs. The above strong and wide absorption of PEI-Ce6/PSS-Au nrs in the wavelength of 700–1000 nm might produce good photothermal effects. To prove this, under 808 nm laser irradiation (1.0 W cm\(^{-2}\), 10 min), temperature of the systems containing PSS-Au nrs, PEI-Ce6, PEI-Ce6/PSS-Au nrs were monitored every 2 min respectively. In Fig. 4A, for the PEI-Ce6/PSS-Au nrs and PSS-Au nrs system, the temperature raise rapidly up to 57 °C after 10 min of laser irradiation, while in the case of PEI-Ce6, slight temperature increase was observed, which was nearly similar with that of pure water. The same results were recorded and shown in Fig. 4B when under dual-wavelength irradiation of 660 nm and 808 nm. These results proved that the PEI-Ce6/PSS-Au nrs composite had a high heat conversion from laser irradiation and PSS-Au nrs played a key role in the conversion.

Since ROS are primarily responsible for cell death in PDT modality, the estimation of ROS production is a key point. Usually, ABDA is used as an indicator to measure the production of ROS. Here, by this way, ROS production in PSS-Au nrs, PEI-Ce6 and PEI-Ce6/PSS-Au nrs induced by the 660 nm laser irradiation (0.5 W cm\(^{-2}\)) was determined every 2 min until 20 min, respectively. As shown in Fig. 5A, PEI-Ce6 and PEI-Ce6/PSS-Au nrs showed obvious decline in ABDA absorption in the range of 300–425 nm during the irradiation, which means the rapid production of ROS. While in the case of PSS-Au nrs, there is no change in ABDA absorption (Fig. 5B), even being irradiated with dual-wavelength laser of 660 nm and 808 nm. Therefore, the PEI-Ce6/PSS-Au nrs showed good photodynamic activity by producing rapidly ROS under the 660 nm laser irradiation mainly due to the contribution of PEI-Ce6.

The excellent photothermal effects and high photodynamic activity of the PEI-Ce6/PSS-Au nrs encourage us to evaluate its feasibility as a PTT/PDT dual modal agent for cancer phototherapy. Cytotoxicity is always the first evaluation object for any biomedical materials. Here, the human squamous cell carcinoma cells were taken as cell models to investigate the cytotoxicity of the PEI-Ce6/PSS-Au nrs. Obviously, all the studied systems including PSS-Au nrs, PEI-Ce6 and PEI-Ce6/PSS-Au nrs showed a very low cytotoxicity in the absence of laser irradiation, in which the cells remained more than 90% alive after 24 h of incubation (Fig. 6A). When being irradiated by the 660 nm laser (0.5 W cm\(^{-2}\), 10 min), the cell viability decreased to 71.1% and 59.8% for PEI-Ce6 and PEI-Ce6/PSS-Au nrs, respectively, due to the production of ROS. Simultaneously, when using the 808 nm laser irradiation (1.0 W cm\(^{-2}\), 10 min), it was found that PSS-Au nrs and PEI-Ce6/PSS-Au nrs showed comparatively high cytotoxicity, and the cell viability decreased to 60.0% and 53.1%, respectively. Most significantly, upon the combined irradiation of both lasers, the cells viability of the cells cultured on the PEI-Ce6/PSS-Au nrs was dramatically decreased and only 19.0% of that

<table>
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<th>Systems</th>
<th>Zeta-potential (mV)</th>
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<tr>
<td>Ce6</td>
<td>−5.50 ± 1.1</td>
</tr>
<tr>
<td>PEI-Ce6</td>
<td>32.2 ± 4.2</td>
</tr>
<tr>
<td>Au nrs</td>
<td>41.3 ± 4.4</td>
</tr>
<tr>
<td>PSS-Au nrs</td>
<td>−54.4 ± 5.1</td>
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**Table 1**

Zeta potentials of Ce6, PEI-Ce6, Au nrs and PSS-Au nrs.

**Fig. 1.** A) UV–vis absorption spectra and B) FTIR spectra of Ce6 and PEI-Ce6.

**Fig. 2.** A) UV–vis absorption spectra of Au nrs and PSS-Au nrs. B) TEM image of PSS-Au nrs.
survived, which is the lowest cell viability among all the studied systems. Taken together, all these results proved the excellent PTT/PDT combinational effects of the PEI-Ce6/PSS-Au nrs. We used Calcine AM (Ca AM) and PI to co-stain the cells to assess the viability of the cells treated with all the above systems (Fig. 6B). Green fluorescence suggested the cells alive and the red dead. In the control group and all the groups without laser irradiation, the cells were almost all in green. While upon laser irradiation, in the PDT and PTT group, some cells were killed. Most importantly, in the PEI-Ce6/PSS-Au nrs groups, the highly intense red fluorescence implied almost all the cells dead. These results were in good agreement with that of the CCK-8 assay.

Next, we further investigated the combinational therapy effects of the PEI-Ce6/PSS-Au nrs in vivo using the squamous cell carcinoma-bearing mice. The mice were divided into 5 groups including group I: negative control; group II: positive control, the mice being treated with blank nonwoven fabric; group III: those being treated with PEI-Ce6; group IV: those being treated with PSS-Au nrs; group V: those being treated with PEI-Ce6/PSS-Au nrs. In each group, they are parted into two subgroups, one received laser irradiation of the 660 nm, or the 808 nm, or both, and the other did not receive any irradiation. For all the 5 groups, the tumors volume of the mice were monitored for 16 days and recorded in Fig. 7A.

As shown in the figure, there was rapid growth of tumor volumes in subgroup I-0, I-1, II-0, II-1, III-0, IV-0, V-0, especially in I-1 and II-1 receiving laser irradiation, indicating that only laser irradiation without phototherapeutic agents had no effect on the tumor growth. While for the mice in the subgroup III-1 and IV-1, they experienced an obvious delayed tumor growth during the treatment with PEI-Ce6 under the 660 nm laser irradiation and with PSS-Au nrs under the 808 nm laser irradiation, respectively, meaning that the PDT or PTT treatment could suppress the growth of tumors obviously. Most notably, in the case of subgroup V-1, the tumors could be destroyed completely after 16 days without recurrence for 60 days. These results clearly demonstrated the superiority of the combinational PDT/PTT over any single modality treatment. Additionally, the photos of the mice treated with the PEI-Ce6/PSS-Au nrs were taken by digital camera and shown in Fig. 7B.

To evaluate the in vivo toxicity of the therapeutic plaster, we monitored the body weight of the mice during the treatment. For all groups, no obvious weight loss was observed in Fig. 8, implying that the toxicity or side effects of all the studied systems are not serious.

4. Conclusions

A PDT/PTT combinational therapeutic plaster based on PEI-Ce6/PSS-Au nrs composite was fabricated. The cancer treatment was induced by using dual-wavelength laser irradiation (660 nm and 808 nm). The plaster could be irradiated repeatedly during the treatment and was completely noninvasive. It has shown its high photothermal efficiency and high ROS production. Both in vitro cytotoxicity and in vivo antitumor effects evaluation demonstrated that the combinational therapy in the PEI-Ce6/PSS-Au nrs was much more efficient.
Fig. 5. A) Absorptions of ABDA in the presence of the PEI-Ce6/PSS-Au nrs under the 660 nm laser irradiation at different time points. B) Normalized absorption of ABDA at 400 nm at different irradiation time in the presence of PEI-Ce6, PSS-Au nrs, and PEI-Ce6/PSS-Au nrs, respectively. The irradiation results under the 660 nm laser and those under the 660/880 nm dual-wavelength laser were shown together. The water was used as a control.

Fig. 6. A) Cytotoxicity of the cells cultured on TCPS, blank nonwoven substrate, PEI-Ce6, PSS-Au nrs and PEI-Ce6/PSS-Au nrs receiving the 660 nm laser (0.5 Wcm$^{-2}$, 10 min), or the 808 nm laser (1.0 Wcm$^{-2}$, 10 min), or both and receiving no any irradiation. B) Fluorescence images of the cells co-stained with Ca AM and PI cultured on blank nonwoven substrate, PEI-Ce6 (PDT), PSS-Ce6 (PTT), and the PEI-Ce6/PSS-Au nrs (PDT + PTT) receiving laser irradiation or receiving no laser irradiation. All the scale bars are 200 μm.

Fig. 7. A) The relative tumor volume changes for the mice in 5 different groups during the treatment of 16 days. I: negative control; II: positive control, with blank nonwoven fabric; III: with PEI-Ce6; IV: with PSS-Au nrs; V: with PEI-Ce6/PSS-Au nrs. 0: laser off; 1: laser on. III-1: PDT; IV-1: PTT; V-I: PDT + PTT. B) Photos of the mice treated with the PEI-Ce6/PSS-Au nrs at different days.
than any single modality treatment. Therefore, the PEI-Ce6/PSS-Au nrs nanocomposite plaster might be a promising therapeutic strategy for cancer treatment.

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