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# Plasmonic titanium nitride nanoparticles for *in vivo* photoacoustic tomography imaging and photothermal cancer therapy

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ABSTRACT

Titanium nitride, an alternative plasmonic material to gold with unique physiochemical properties, has been widely used in microelectronics, biomedical devices and food-contact applications. However, its potential application in the area of biomedicine has not been effectively explored. With the spectral match of their plasmon resonance band and the biological transparency window as well as good biocompatibility, titanium nitride nanoparticles (TiN NPs) are promising photoabsorbing agents for photothermal therapy (PTT) and photoacoustic imaging. Nevertheless, the photothermal performance of TiN NPs has not been investigated until now. Here, we presented the investigation of employing TiN NPs as photoabsorbing agents for *in vivo* photoacoustic tomography (PAT) imaging-guided photothermal cancer therapy. Our experimental results showed that TiN NPs could strongly absorb the NIR light and provided up to 48% photothermal conversion efficiency. After PEGylation, the resultant nanoparticles demonstrated improved physiological stability and extensive blood retention. Following intravenously administration, they could simultaneously enhance the photoacoustic signals of the tumor region and destroy tumors in the tumor-bearing mouse model by taking advantage of the photothermal effect of the TiN NPs. Our findings highlighted the great potential of plasmonic TiN NPs in detection and treatment of cancer.



## 1. Introduction

Plasmonic nanomaterials with localized surface plasmon resonances in the near infrared (NIR) region have been extensively explored as photoabsorbing agents for photothermal cancer therapy over the past decades [1,2]. As compared to conventional photoabsorbing dyes, plasmonic nanoparticles not only possess stronger NIR light absorption originating from the surface plasmon-enhanced light-matter interaction but also exhibit higher photostability [2]. Gold nanostructures have long been used as photoabsorbents for plasmon-mediated photothermal therapy (PTT) owing to their biocompatibility and relatively good plasmonic properties [3–5]. However, the plasmon resonances peaks of gold nanoparticles occur at the shorter wavelength outside the biological window (700–1000 nm). In order to achieve efficient NIR light absorption, researchers have to redshift their resonance bands into the biological window by using complex structures (such as gold nanorod, nanoshell). Moreover, gold is a noble metal with high price and the complex nanostructures can not only lead to the instability problems of the nanoparticles upon light irradiation but also affect their pharmacokinetics *in vivo* [6–8]. Although many cost-effective nanoparticles with favorable photothermal performance such as carbonaceous nanomaterials [9–11], polymeric nanoparticles [12–14], copper-based semiconductors [15–17], magnetic iron oxide nanoparticles [18,19], two-dimentional transition metal-based nanomaterials [20–24], black phosphorous quantum dots [25,26], tungsten oxide quantum dots [27], and bismuth-based nanoparticles [28] et al. were successively employed for photothermal tumor therapy, many of these nanomaterials still suffered from limitations including low photothermal conversion efficiency and complicated synthesis processes. Therefore, the development of new photothermal agents with high efficiency, facile synthesis procedures, cost-effectiveness and abundant availability is of scientific and clinical interest.

Titanium nitride is a contamination-safe material with good biocompatibility and has been widely used in surgical tools,

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implants and food-contact applications [6]. As a semimetal, titanium nitride is also a promising plasmonic material and has recently been proposed as an alternative to gold for plasmonic applications due to their unique physicochemical properties and lower cost [7]. Distinct from gold nanoparticles, the localized surface plasmon resonance band of titanium nitride nanoparticles (TiN NPs) is just located within the biological transparency window, thus they can strongly absorb the NIR light without the need for complex structures [7,29]. Moreover, TiN NPs is an inexpensive material and commercially available in large amounts (The comparison of the cost of TiN NPs and some other PTT agents previously reported was supplied in Supporting Information Table S1). On the basis of the above merits, TiN NPs hold great promise to be an excellent photothermal agent for photothermal cancer therapy. However, the report of exploring TiN NPs for biomedical applications is still quite rare, especially in the area of cancer treatment.

In this work, we studied the performance of plasmonic TiN NPs as photothermal agents for PTT, and their utilization as photoacoustic tomography (PAT) imaging contrast agents for *in vivo* tumor detection was investigated as well. TiN NPs had several advanced features that made them highly superior for utilization in cancer diagnosis and therapy. First, they could strongly absorb the NIR light and displayed a high photothermal conversion efficiency of 48%, as well as good photostability. These features rendered TiN NPs both the local heating capability for photothermal cancer therapy and the imaging ability to enhance the photoacoustic signals. Second, by taking advantage of the self-passivating native oxide at the surface, they could be easily modified with biocompatible agents to improve their stability and biocompatibility for *in vivo* applications. Third, following intravenously injection, these nanoparticles could effectively home in the tumor region through the enhanced permeation and retention (EPR) effect, which was visualized by PAT imaging. Upon NIR light irradiation, they were able to induce complete tumor regression in the observation time periods. Our work highlighted that plasmonic TiN NPs with appropriate surface modification (PEGylation) were promising photoabsorping agents for PAT imaging-guided cancer therapy.

## 2. Materials and methods

### 2.1. Synthesis of TiN-PEG NPs

PEGylated TiN NPs was synthesized through the silane-coupling chemistry. Briefly, TiN NPs 1.0 g TiN NPs (purchased from Shanghai Chaowei Nano Technology Co., Ltd.) was dispersed into pure water (200 mL) and centrifuged (8000 rpm, 3 min) to remove large aggregates. The resultant dispersions were further condensed to a concentration of about 10 mg $mL^{-1}$ (8 mL) and transferred to a round bottomed flask, mPEG-silane (0.1 g) dissolved in ethanol/water (2 mL, w/w, 95%/5%) mixed solution was then added. The mixture was stirred at room temperature (25 °C) for 2 h to complete the reaction. The obtained solution was transferred into dialysis bag (MW = 8000–14000) and dialyzed against pure water for 24 h to remove the residual mPEG-silane. After freeze-drying, the obtained TiN-PEG NPs was stored at argon atmosphere for further use.

### 2.2. Synthesis of Au nanorods (Au NRs)

Au NRs were prepared using the silver ion-assisted seed-mediated method reported previously [30]. Typically, the seed solution was synthesized by the addition of $HAuCl_4$ (0.01 mol $L^{-1}$, 0.25 mL) into cetyltrimethylammonium bromide (CTAB, 0.1 mol $L^{-1}$, 10 mL) under mild stirring. Then, a freshly prepared, ice-cold $NaBH_4$ solution (0.01 mol $L^{-1}$, 0.6 mL) was added into the mixture solution with vigorous stirring for 2 min. The seed solution was left undisturbed for 4 h at room temperature. To grow Au nanorods, $HAuCl_4$ (0.01 mol $L^{-1}$, 2.0 mL) and $AgNO_3$ (0.01 mol $L^{-1}$, 0.4 mL) was added into CTAB solution (0.1 mol $L^{-1}$, 40 mL) with gentle mixing, followed by the addition of HCl (1.0 mol $L^{-1}$, 0.8 mL) and ascorbic acid (0.1 mol $L^{-1}$, 0.64 mL) solution. Finally, the seed solution (96 μL) was added dropwise to the growth solution, and the solution was mildly stirred for 30 s and incubated without disturbance at 30 °C for 6 h. The obtained solution was washed with water by centrifugation to eliminate excess CTAB, and then redispersed in water.

### 2.3. Photothermal effect measurement

To study the photothermal effect of TiN NPs, different concentrations of TiN-PEG NPs (1 mL) were irradiated by an NIR laser (808 nm, 2 W $cm^{-2}$) for 10 min, respectively. The temperature of the solutions was monitored every 10 s by a digital thermometer with a thermocouple probe submerged in the solution in a square cuvette. The influence of laser power density on the photothermal effect of TiN NPs was investigated by tracking the temperature variation of TiN-PEG NPs aqueous solutions in different power density using an IR camera.

### 2.4. Cell culture and cytotoxicity assay

HeLa cells were cultured in DMEM medium (Gibco) and 4T1 cells were cultured in RPMI-1640 medium (Gibco) both with 10% fetal bovine serum (FBS, Gibco) at 37 °C in a humidified atmosphere with 5% $CO_2$. Cell viability was measured by the methyl thiazolyl tetrazolium (MTT) assay. Typically, HeLa cells (4T1 cells) were seeded in 96-well plates and then incubated with varying concentrations of TiN-PEG NPs (100 μL) for 24 h at 37 °C in a humidified 5% $CO_2$ atmosphere, and then the culture medium was removed and washed with PBS twice. The freshly prepared culture medium containing MTT reagent (10%, 100 μL) was added to each well of the 96-well plate and incubated for 4 h to allow formation of formazan dye. After incubation, the medium was removed and DMSO (200 μL) was added. Then the 96-well plate was placed into the enzyme-linked immunosorbent assay reader, and shaked for 15 min to allow the dissolution of formazan. Finally, the optical absorption of formazan at 570 nm was measured.

### 2.5. Photothermal cytotoxicity

4T1 (HeLa) cells were incubated in cell culture dish at 37 °C containing TiN-PEG NPs (100 μg $mL^{-1}$) for 4 h, and then the TiN-PEG NPs solution was removed and washed with PBS solution twice. After that, the cells were irradiated under the 808 nm NIR laser light at a power density of 2 W $cm^{-2}$ for 5 min. After irradiation, the cells were co-stained with calcein acetoxymethyl ester (calcein AM) and propidium iodide (PI) for 15 min, washed with PBS twice, and then imaged by confocal laser microscope.

To quantitatively evaluate the photothermal cytotoxicity of TiN-PEG NPs, 4T1 (HeLa) cells were incubated in 96-well plates at 37 °C in a humidified atmosphere containing 5% $CO_2$ for 12 h, and then TiN-PEG NPs with different concentrations were added. Next, the cells were further incubated for 4 h and the culture medium was removed and washed with PBS twice. Thereafter, the cells were exposed to the 808 nm NIR laser at a power density of 2 W $cm^{-2}$ for 5 min and then incubated for another 24 h. The viability and proliferation of both 4T1 (HeLa) cells were measured by the MTT assay.