880 nm NIR-triggered organic small molecular based nanoparticles for photothermal therapy of tumor

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March 30, 2022

Abstract

Photothermal therapy (PTT) has received constant attention as efficient cancer therapy method due to locally selective treatment and not affected by tumor microenvironment. In this study, a novel 880 nm near-infrared (NIR) laser triggered photothermal agent (PTA), 3TT-IC-4Cl, was used for PTT of tumor in deep tissue. Folic acid (FA) conjugated amphiphilic block copolymer (folic acid-polyethylene gly-col-poly (β-benzyl-L-aspartate)10, FA-PEG-PBLA10) was employed to encapsulate 3TT-IC-4Cl by nano-precipitation to form stable nanoparticles (TNPs), TNPs exhibit excellent photothermal stability and photothermal conversion efficiency. Furthermore, the in vitro results showed TNPs display excel-lent biocompatibility and significant phototoxicity. These results suggest that 880 nm triggered TNPs has great potential as an effective PTAs for photothermal therapy of tumor in deep tissue.

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ABSTRACT: Photothermal therapy (PTT) has received constant attention as efficient cancer therapy method due to locally selective treatment and not affected by tumor microenvironment. In this study, a novel 880 nm near-infrared (NIR) laser triggered photothermal agent (PTA), 3TT-IC-4Cl, was used for PTT of tumor in deep tissue. Folic acid (FA) conjugated amphiphilic block copolymer (folic acid-polyethylene glycol-poly (β-benzyl-L-aspartate)$_{10}$, FA-PEG-PBLA$_{10}$) was employed to encapsulate 3TT-IC-4Cl by nano-precipitation to form stable nanoparticles (TNPs), TNPs exhibit excellent photothermal stability and photothermal conversion efficiency. Furthermore, the in vitro results showed TNPs display excellent biocompatibility and significant phototoxicity. These results suggest that 880 nm triggered TNPs has great potential as an effective PTAs for photothermal therapy of tumor in deep tissue.

KEYWORDS : photothermal therapy, NIR-triggered, photothermal agent, deep tissue, nanoparticles

1. Introduction

Phototherapy has attracted extensive attention in recent years as a powerful cancer treatment method due to their characteristics such as convenience, noninvasiveness, locally selective treatment, negligible drug resistance and minimized adverse side effect[1]. Photodynamic therapy (PDT) and photothermal therapy (PTT) are two typical phototherapy approaches, PTT is based on the photothermal agents (PTA) which

are preferentially taken up and retained by diseased tissue, then after excitation by appropriate wavelength laser, the PTA convert light to heat to induce cancer cells apoptosis or necrosis. Compare to PDT, PTT is not affected by tumor microenvironment such as the local oxygen level, so PTT have received increasing attention and develop rapidly in recent years.

PTAs are one of the most important factors determining the efficiency of PTT, many kinds of PTA have been developed in recent years. Current PTA can be classified as inorganic and organic materials, compare to inorganic PTA, the organic PTA with easy chemical structure tuning, good biocompatibility, low-toxicity, and easy metabolism in biological system are more desirable for clinical phototheranostics[2-7], such as cyanine days[8-11], diketopyrrolopyrrole derivatives[12, 13], croconaine-based agents[14, 15], porphyrin-based agents[16-19], conjugated polymers[20-22], squaraine derivatives[23, 24], boron dipyrromethane (BODIPY) dyes[25] and so on. In organic PTAs, the polymeric PTA was limited due to their complicated fabrication processes, indistinct biodegradation, and potential biosafety[19]. So, the small organic molecules have received increasing attention as potential alternatives to nanomaterials in the area of PTT recently.

In addition, another main challenge for phototherapy is efficient treat cancers at a deep tissue level. Near-infrared (NIR) light is referred to as the "optical window" of the biological tissues due to the minimal light absorption and scattering. Compared with the UV or visible light, NIR shows larger penetration distance in tissue, lower photodamage effect and higher signal-to-noise ratio [26],[27]. The organic molecules with extended π-conjugation usually show strong NIR absorbance, which is beneficial for deep tumor tissue diagnosis and phototherapy[28-30]. The well designed conjugated small molecules organic PTA, especially recently reported acceptor-donor-acceptor (A-D-A) structure PTAs would open a new gate for efficient PTT of tumor in deep tissues [31-33].

However, a problem limiting the use of conjugated small molecules organic PTA is their low water solubility, the hydrophobic PTA difficult to prepare pharmaceutical formulations and cannot be directly injected intravenously. To overcome these problems, various strategies have been employed to prepare water-soluble and stable formulations of hydrophobic organic PTA, such as conjugate to water-soluble polymers[11], loaded into mesoporous materials[17] or carbon materials[34-36], encapsulate in colloidal carriers such as liposomes[16], polymer nanoparticles[9, 10, 12, 13, 18-22, 25, 37, 38].

In this study, an A-D-A structure non-fullerene molecules, 3TT-IC-4Cl, which includes three fused thieno[3,2-b]thiophene as the central core and difluoro substituted indanone as the end groups was selected as PTA for PTT. Similar with other A-D-A structure non-fullerene molecules, 3TT-IC-4Cl exhibits both broad absorption and effectively suppressed fluorescence[32], especially, 3TT-IC-4Cl exhibit strong and broad absorption in 800-900 nm regions after forming nanoparticles, it's indicated that the 3TT-IC-4Cl has the potential as PTA for NIR-triggered PTT of cancer in deep tissue. In order to effectively utilize 3TT-IC-4Cl for PTT, herein, our previous reported folic acid (FA) conjugated amphiphilic block copolymer (folic acid-polyethylene glycol-poly (β-benzyl-L-aspartate)$_{10}$, FA-PEG-PBLA$_{10}$) was employed to encapsulate 3TT-IC-4Cl by nano-precipitation and dialysis process to form stable nanoparticles (TNPs), and improve 3TT-IC-4Cl solubility in aqueous solution. In the TNPs system, 3TT-IC-4Cl and PBLA segment of copolymer as inner core for 3TT-IC-4Cl storage, 3TT-IC-4Cl as the heat source, and PEG segment as the out shell to improve solubility, stability and biocompatibility of this system, active targeting ligand FA was introduced to the surface of nanoparticles to enhance selectivity of nanoparticles.

Recently, the NIR-triggered organic small molecular based PTT system have been developed[9, 10, 12, 13, 17, 22, 25, 39], however, few systems of A-D-A type small molecular organic PTA based and 880 nm triggered PTT have been reported.

2. Results and Discussion

2.1 Synthesis and Characterization of TNPs

A novel PTAs with 880nm triggered A-D-A structure non-fullerene molecules, 3TT-IC-4Cl, which includes three fused thieno[3,2-b]thiophene as the central core and difluoro substituted indanone as the end groups[40]

was selected for PTT. In order to effectively utilize 3TT-IC-4Cl for tumor therapy. An amphiphilic block copolymer (FA-PEG-PBLA$_{10}$) was synthesized as our previous reported method[41] and used for 3TT-IC-4Cl encapsulation, 3TT-IC-4Cl was encapsulated in FA-PEG-PBLA$_{10}$ by nano-precipitation and dialysis process to form stable nanoparticles (TNPs), as shown in Figure 1, PBLA segment of copolymer as a reservoir for 3TT-IC-4Cl storage in the inner core, PEG segment as the out shell to improve solubility, stability and biocompatibility of TNPs, active targeting ligand FA was introduced to the surface of nanoparticles to enhance selectivity of nanoparticles, the chemical structure was confirmed by ^{1}H NMR, as shown in Figure 2A, the characteristic peaks a and b are belong to FA-PEG-PBLA$_{10}$, and the characteristic peaks c, d, e, f, g, and h attribute to 3TT-IC-4Cl, respectively. It indicated that the 3TT-IC-4Cl was encapsulated in FA-PEG-PBLA$_{10}$successfully, the encapsulation rate (93.5%) was calculated by the relative intensity ratio of the methylene proton of PEG at 3.5 ppm and the proton of the alkane chain of in 3TT-IC-4Cl at about 1 ppm.

For nanomedicine used in cancer therapy, the size, morphology, and stability are the key properties that influence in vivo performance. These factors affect the biodistribution and circulation time of the drug carriers. Stable and smaller particles have reduced uptake by the RES and provide efficient passive tumor-targeting ability via an enhanced permeation and retention (EPR) effect[42]. The morphology of TNPs was evaluated by TEM, as shown in Figure 3. The TNPs were submicron in size and uniform nearly spherical with no aggregation between nanoparticles observed due to the polymer modification, the average diameter was 150 nm. DLS measurements showed average hydrodynamic diameter of TNPs is about 200 nm (Figure 3, inset), a suitable size for passive targeting ability through EPR effect. The size distribution of TNPs maintained a narrow and monodisperse unimodal pattern.

2.2 Optical properties of TNPs

The optical properties of TNPs was investigated by UV-vis absorption spectra and fluorescence spectra (Figure 4A and Figure 4B), for free 3TT-IC-4Cl in $CHCl_3$ solution, it shows strong absorption at 772 nm and a maximal fluorescence at about 840 nm. However, after formation nanoparticles, the TNPs aqueous solution exhibit strong absorption at 874 nm, the significant red shift would due to the π-π stacking of 3TT-IC-4Cl during the nanoparticles formation, this result would conducive to trigger TNPs by 880 nm NIR light source for phototherapy of tumor in deep tissue. In the other hand, compare to free 3TT-IC-4Cl in $CHCl_3$ solution, the TNPs aqueous solution nearly no fluorescence signal is observed due to the 3TT-IC-4Cl aggregation during the nanoparticles formation, which would significantly increases nonradiative heat generation, and enhance PTT efficiency[18, 43].

2.3 Photothermal Properties of TNPs *in Vitro*

To investigate the photothermal conversion property of the TNPs, the temperature of TNPs aqueous solution with a series of concentrations (from 0 to 250 μg/mL) under the 880 nm laser irradiation (0.7 W/cm^2) for 15 min was monitored (Figure 5A), the related infrared (IR) thermal images of TNPs aqueous solution were showed in Figure 5C. As shown in the Figures, the temperature increased significantly as TNPs concentration increased. It is noted that the TNPs at 90 μg/mL exhibit effective hyperthermia (>50), which is sufficient to induce apoptosis or necrosis of cancer cells[44]. The relationship between temperature of TNPs aqueous solution (180 μg/mL) and different laser power (from 0.3 to 1.5 W/cm^2) was future measured, as shown in Figure 5B, the temperature of the TNPs aqueous solution depends on the laser power. The related infrared (IR) thermal images of TNPs aqueous solution were showed in Figure 5D. In the other hand, we also investigated the photothermal conversion efficiency of TNPs through a cycle of heat-up and cooling using previous reported method (Figure S1)[45]. The photothermal conversion efficiency of the TNPs was 31.5%, which is more higher than other PTAs such as cyanine dyes (e.g.,[?]26.6%) and gold nanorods (e.g.,[?]21.0%)[22, 46, 47]. The strong absorption and high photothermal conversion efficiency of TNPs in the NIR region provided the potential of photothermal treatment of cancer.

2.4 Photothermal stability of TNPs

The photothermal stability is an important parameter of photothermal drugs for PTT applications, it would

be crucial for clinical application and therapeutic efficiency. The photothermal stability of TNPs was evaluated by monitoring its ability to maintain the temperature elevation. As shown in Figure 6A, the TNPs was irradiated at 0.7 W/cm^2 for 5 min, then the laser was turned off, following the samples were cooling down to the room temperature, the temperature was recorded by IR thermal camera throughout the process, this irradiation/cooling procedures were repeated five times, as Figure 6A shows, TNPs displayed negligible change in their temperature elevation after five irradiation/cooling cycles. However, the temperature elevation of free ICG decreased significantly after one irradiation/cooling cycle. In the other hand, we also observed the changes in the color of samples, as shown in Figure 6B, after 5 min irradiation, the color of free ICG solution changed observably, but the TNPs exhibit no change after 30 min irradiation. These results indicated the TNPs exhibit excellent photothermal stability.

2.5 *In Vitro* Cell Test

In order to investigate the feasibility of TNPs as nano photothermal agents for PTT, the *in vitro* cytotoxicity of TNPs was investigated by MTT assay and the average cell viability was monitored. For biocompatibility test, the dark toxicity of TNPs was investigated. As shown in Figure 7A, both FA-PEG-PBLA$_{10}$ and TNPs exhibited no significant dark toxicity. As the concentration increased, the average cell viability was greater than 90% even when cells were treated with 250 μg/mL of TNPs. For the phototoxicity test, we investigated the concentration dependent (0, 30, 60, 90, 120, 180, and 250 μg/mL) cytotoxicity of TNPs with 880 nm laser irradiation. As shown in Figure 7B, after irradiation at 0.7 W/cm^2 for 5 min, the cell viability gradually decreased as the TNPs concentration increased. Taken together, these results indicate that the TNPs could considerably enhance the efficiency of PTT for tumor in deep tissue, even at low concentrations.

3. Conclusions

In summary, an 880 nm NIR laser triggered TNPs as PTA for photothermal therapy of tumor in deep tissue was developed. In this work, a novel PTA, 3TT-IC-4Cl, was selected and used for PTT, it includes three fused thieno[3,2-b]thiophene as the central core and difluoro substituted indanone as the end groups, after encapsulation by FA-PEG-PBLA$_{10}$ block copolymer and forming nanoparticles, the TNPs aqueous solution exhibit strong absorption at 880 nm due to the π-π stacking. DLS and TEM measurements showed that the TNPs have spherical shape and narrow size distribution with a mean diameter of 150 nm. These stable nanoparticles are suitable for the EPR effect and accumulation in the tumor tissue. TNPs exhibit excellent photothermal stability and high photothermal conversion efficiency after 880 nm laser irradiation. In the *in vitro* test, TNPs display excellent biocompatibility and significant phototoxicity. Therefore, the 880 nm triggered TNPs has great potential as an effective PTAs for photothermal therapy of tumor in deep tissue.

4. Experimental Section

4.1 Materials

Folic Acid (FA), PEG-bis(amine) (Mn: 3.4 kDa), β-benzyl-L-aspartate (BLA), Triethylamine (TEA), MTT, PBS, and Sodium Bicarbonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Triphosgene was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). N-hydroxysuccinimide (NHS) and N, N'-dicyclohexylcarbodiimide were purchased from Fluka (Buchs, Switzerland). 3TT-IC-4Cl was provided by SunaTech Inc. (Suzhou, China). Indocyanine Green (ICG) was purchased from Adamas (Shanghai, China). CHCl3 was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). DMSO was purchased from Fuchen Chemical Reagent Co.,Ltd (Tianjin, China). Chloroform-d was purchased from Tenglong Weibo Technology Co., Ltd (Qingdao, China). DMSO-d_6 was purchased from Ningbo Cuiying Chemical Technology Co., Ltd (Ningbo, China). Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), Penicillin, and Streptomycin were purchased from GibcoBRL (Invitrogen Corp., CA, USA). All other chemicals were of analytical grade and used as received without further purification.

4.2 Characterization

The chemical structure was determined by 400 MHz ^{1}H NMR (AVANCE III HD 400MHz, Bruker, Fällanden, Switzerland) using $CHCl_3$-d and DMSO-d_6 as the solvent. The photophysical properties of samples in aqueous

solution were confirmed by UV-visible spectrophotometry (UV-2550, Shimadzu, Tokyo, Japan) and fluorescence spectrophotometer (F-4600, Hitachi, Tokyo, Japan). The morphologies, sizes, and size distributions of nanoparticles were determined by transmission electron microscopes (TEM) (TECNAI G2 Spirit TWIN, FEI, Hillsboro, FL, USA) and dynamic light scattering (DLS) (Zetasizer Nano ZS90, Malvern Instruments Co, Malvern, UK) at 25^{o}C using a He-Ne laser (633 nm) as a light source. The temperature was monitored by IR thermal camera (TiS65, Fluke, Everett, WA, USA). The NIR laser (880 nm) used in this study was purchased from Beijing Laserwave Optoelectronics Technology Co., Ltd. (LWIRL880-20W-F, Laserwave, Beijing, China).

4.3 Preparation of TNPs

In order to prepare TNPs, first, the amphiphilic block copolymer FA-PEG-PBLA$_{10}$ used for 3TT-IC-4Cl encapsulation was synthesized by ring-opening polymerization as our previous reported[41]. The chemical structure of FA-PEG-PBLA$_{10}$ was confirmed by ^{1}H NMR (400 MHz, DMSO). Then, the TNPs were prepared by nanoprecipitation method. Briefly, 5 mg 3TT-IC-4Cl was dissolved in 1 mL THF, then, the 3TT-IC-4Cl solution was added into to 50 mL FA-PEG-PBLA$_{10}$ solution (0.5 mg/mL in DMSO) dropwise, and then the mixture was transfer to dialysis tubs (Cut-off 3.5 K Mw) to remove THF and DMSO, followed by freeze drying, the TNPs were obtained.

3.4 Photothermal Effect

To confirm the PTT application potential, the photohermal property of TNPs was investigated, a series concentrations of TNPs (0, 30, 90, 180, and 250 μg/mL) in water were irradiated by 880 nm laser (0.7 W/cm^2) for 720 s, the temperature of TNPs solution was recorded by IR thermal camera every 30 s. In addition, the constant concentration (180 μg/mL) of TNPs were irradiated by 880 nm laser for 720 s with various power densities (0.3, 0.5, 0.8, and 1.5 W/cm^2) was investigated by same method.

3.5 Stability of TNPs

In order to investigated the stability of TNPs, TNPs (180 μg/mL, 30 μg/mL free 3TT-IC-4Cl equiv.), and free ICG (30 μg/mL) were irradiated with 880 nm laser (0.7 W/cm^2) for 5 min, then the laser was turned off and the sample was cooled to the room temperature naturally, the temperature of samples were recorded using the IR thermal camera every 30 s. Subsequently, the procedures were repeated four times.

3.6 *In vitro* Phototoxicity and Biocompatibility of TNPs

HeLa cells (1×10^4 cells/well) were seeded onto 96-well plates in 200 μl DMEM and allowed to attach for 24 h. After cell attachment, the medium was replaced with 100 μl of fresh medium containing FA-PEG-PBLA$_{10}$ and TNPs with a series of concentration (0, 30, 60, 90, 120, 180, and 250 μg/mL), and then incubated for 4 h. The cells were washed with PBS and replace with fresh DMEM. The samples were irradiated with a laser (880 nm, 0.7 mW/cm^2) for 5 min. Then, irradiated cells were incubated at 37^{o}C for 24 h and cell viability was evaluated by MTT assay. Data presented are averaged results of quadruplicate experiments. For biocompatibility, HeLa cells (1×10^4 cells/well) were seeded onto 96-well plates in 200 μl DMEM and allowed to attach for 24 h. After cell attachment, the medium was replaced with 100 μl of fresh medium containing FA-PEG-PBLA$_{10}$ and TNPs with a series of concentration (0, 30, 60, 90, 120, 180, and 250 μg/mL), and then incubated for 24 h. The cell viability was evaluated by MTT assay. Data presented are averaged results of quadruplicate experiments.

Supporting Information

The Supporting Information is available free of charge at https://www.elsevier.com

Author Contributions

#Y.Z., and Z.H. contributed equally to this work.**Notes** The authors declare no competing financial interest.

ACKNOWLEDGMENT

This research was supported by the National Natural Science Foundation of China (Grant Nos. 51703163), the Natural Science Foundation of Tianjin (18JCZDJC34600 and 18JCYBJC86700) and the Program for Prominent Young College Teachers of Tianjin Educational Committee.

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Figure Legends

Figure 1	Schematic illustration demonstrating of TNPs formation and PTT effect.
Figure 2	^{1}H NMR spectra of (A) TNPs, (B) 3TT-IC-4Cl, and (C) FA-PEG-PBLA$_{10}$.
Figure 3	(A) TEM image of TNPs, (B) Typical size distributions of TNPs (insert).
Figure 4	(A) UV–Vis absorption spectra of free 3TT-IC-4Cl (red) and TNPs (black), and (B) Fluorescence spectra of free
Figure 5	(A) Photothermal conversion behavior of TNPs at different concentrations (0-250 μg/mL) under 880 nm irradiat
Figure 6	(A) Temperature elevation of TNPs, and free ICG under five irradiation/cooling cycles (under 880 nm irradiatio
Figure 7	In vitro cytotoxicity test using FA-PEG-PBLA$_{10}$ and TNPs against HeLa cells (A) dark toxicity depending on t

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Figure 1

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Figure 2

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Figure 3

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Figure 4

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Figure 5

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Figure 6

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Figure 7

Table of Contents graphic

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