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A dual function theranostic agent for near-infrared photoacoustic imaging and photothermal therapy

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ABSTRACT

Theranostic, defined as combining diagnostic and therapeutic agents, has attracted more attention in biomedical application. It is essential to monitor diseased tissue before treatment. Photothermal therapy (PTT) is a promising treatment of cancer tissue due to minimal invasion, unharmful to normal tissue and high efficiency. Photoacoustic tomography (PAT) is a hybrid nonionizing biomedical imaging modality that combines rich optical contrast and high ultrasonic resolution in a single imaging modality. The near infra-red (NIR) wavelengths, usually used in PAT, can provide deep penetration at the expense of reduced contrast, as the blood absorption drops in the NIR range. Exogenous contrast agents with strong absorption in the NIR wavelength range can enhance the photoacoustic imaging contrast as well as imaging depth. Most theranostic agents incorporating PAT and PTT are inorganic nanomaterials that suffer from poor biocompatibility and biodegradability. Herein, we present an benzo[1,2-c;4,5-c'] bis[1,2,5] thiadiazole (BBT), based theranostic agent which not only acts as photoacoustic contrast agent but also a photothermal therapy agent. Experiments were performed on animal blood and organic nanoparticles embedded in a chicken breast tissue using PAT imaging system at ~803 nm wavelengths. Almost ten time contrast enhancement was observed from the nanoparticle in suspension. More than 6.5 time PA signal enhancement was observed in tissue at 3 cm depth. HeLa cell lines was used to test photothermal effect showing 90% cells were killed after 10 min laser irradiation. Our results indicate that the BBT - based naoparticles are promising theranostic agents for PAT imaging and cancer treatment by photothermal therapy.

***Keyword*:** Photoacoustic imaging, Organic nanoparticles, Contrast agent, Deep-tissue imaging, Pulsed diode laser, Photothermal therapy

1. INTRUDUCTION

Theranostic has attracted great attention since it was coined in 2002.[1] It aims to incorporate various agents into one material, in order to realize imaging and therapy simultaneously. Some of the therapeutic strategies developed for different types of cancer treatments, such as nucleic therapy, chemotherapy, photothermal therapy, photodynamic therapy, and radiation therapy, can be functionalized with imaging strategies, such as magnetic resonance imaging, nuclear imaging, photoacoustic imaging, and fluorescence imaging.[2-4] Out of all, photoacoustic tomography (PAT) is a promising medical imaging technique that combines rich optical contrast and scalable high ultrasonic resolution in a single modality.[5-8] PAT can provide deeper tissue imaging than other pure optical imaging modalities such as fluorescence microscopy, Raman microscopy, Optical coherence tomography, etc [9-12]. PAT has been proven to be a promising technique for imaging biological features from organelle to organs.[5] The application of PAT includes, but not limited to, small animal brain imaging, breast cancer imaging, monitoring of vascularisation, tumor angiogenesis, blood oxygenation, total haemoglobin concentration, etc.[7, 13-15] Biological tissues have relatively low absorption in the near infrared (NIR) region. Therefore, NIR wavelength light has been used for deep tissue PAT imaging. Several exogenous contrast agents with high absorption in the NIR region have been used to enhance the contrast for deep tissue PAT imaging. Several metallic, inorganic, organic nanoparticles, and Quantum dots have been used for deep-PAT imaging.[16-20]

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Among many strategies for cancer treatment, photothermal therapy (PTT) has been widely used due to its advantages such as high specificity, minimal invasiveness, low toxicity to normal tissues, and excellent anti-cancer efficacy.[21-23] As a consequence, many efforts have been devoted to explore various theranostic nanomaterials, especially combining PAT and PTT due to the deep penetration of tissues, high specificity, minimal invasiveness, selective damage and excellent anti-cancer efficacy. Some dual-modal theranostic materials have been reported,[24] such as combining photoacoustic imaging and PTT using inorganic NPs[25] and conjugated polymers.[26] However, these inorganic materials incorporating two functions into one material still suffer from poor biocompatibility and biodegradability.

Benzo[1,2-c;4,5-c']bis[1,2,5]thiadiazole (BBT) based derivatives are well-known narrow-bandgap building blocks for organic optoelectronic devices.[27] Taking the advantage of the strong light absorption of BBT derivatives in near NIR window that can benefit deep tissue imaging and therapy, it is reported that colloidal NPs composed of a small molecular BBT derivative (denoted as BBTEHT) showed high photothermal conversion efficiency and robust photostability compared to gold NPs for effective treatment of cancer cells.[28] In this work, we report a theranostic agent based on a small-molecular BBT derivative, benzo[1,2-c;4,5-c']bis[1,2,5]thiadiazole-4,7-bis(9,9-dioctyl-9H-fluoren-2-yl)thiophene (denoted as BBT-2FT).

2. CHARACTERIZATION OF BBT-2FT NPs

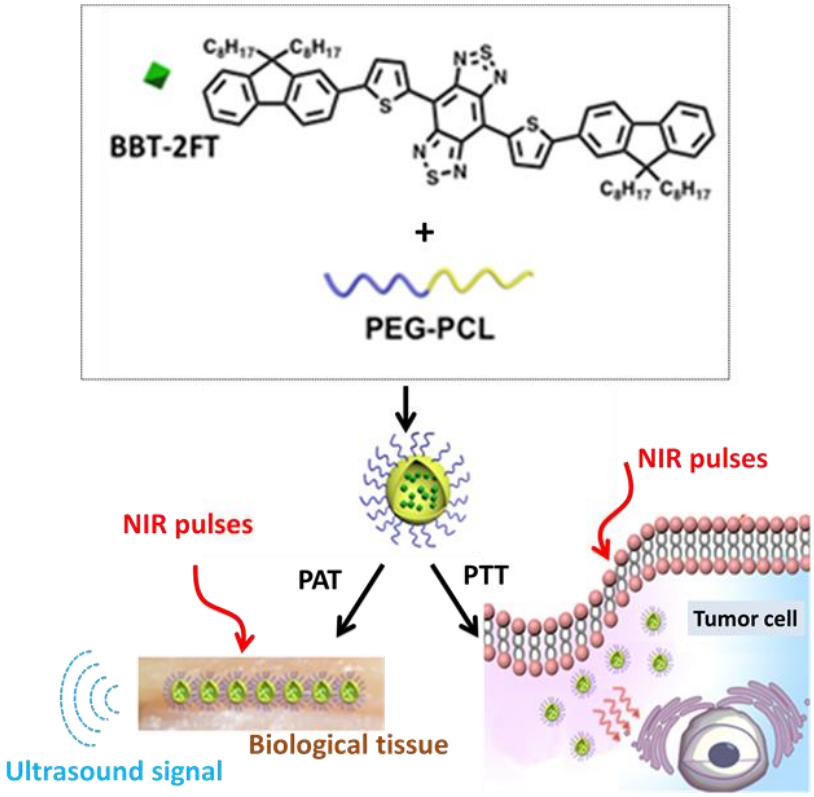

Fig.1. Schematic illustration of the preparation of BBT-2FT nanoparticles and their applications in NIR photothermal therapy and photoacoustic imaging.

Fig. 1 shows the molecular structure of BBT-2FT and a schematic presentation of the NPs formed through a nanoprecipitation process. BBT-2FT molecule was synthesized and confirmed by 1H-NMR, 13C-NMR, and MALDI-TOF mass spectroscopy. The TEM image of the BBT-2FT molecules is shown in Fig. 2a. The molar absorption coefficient of BBT-2FT in toluene at 808 nm was calculated to be $2.7 \times 10^4\ L \cdot Mol^{-1}cm^{-1}$. As shown in Fig. 2b, BBT-2FT shows a strong NIR absorption peak at 880 nm, which is broader compared to that of commercial ICG molecules. The photothermal effect induced by NIR laser illumination at 808 nm with a power density of 1.77 W/cm^2 for 10 min in the presence of BBT-2FT NPs was investigated by monitoring the temperature of 1 mL aqueous dispersion of BBT-2FT NPs at various concentrations (25, 50, and 100 μg/mL). The temperature as a function of time is shown for 25 μg/mL concentration and the control, the pure water as a control showed little change in temperature under the same conditions of laser irradiation. The photothermal conversion efficiency (η) was calculated to be 47%, which is higher than that of the BBT small molecule that we reported previously[28] and those of other reported photothermal agents such as polypyrrole (η = 40%) and Au nanorods (η = 22%).[29]

To further investigate the photostability of BBT-2FT NPs, six cycles of laser ON and OFF with NIR laser were used. The continuous laser (808 nm, 1.77 W/cm^2). Dispersion of BBT-2FT NPs (50 μg/mL) was irradiated with NIR laser for 10 min, followed by naturally cooling (without laser irradiation) to room temperature for 10 min. This cycle was repeated six times in order to investigate the photostability of BBT-2FT NPs. The recorded temperature change indicated no significant photoinduced degradation of BBT-2FT NPs under the present experimental conditions. After 6-cyles, the TEM images showed some morphological changes, however those size changes could not be detected by dynamic light scattering, suggesting that the average size and colloidal stability of the nanoparticles were not significantly affected by the laser irradiation. The promising photothermal conversion efficiency of BBT-2FT NPs prompted us to investigate the application of these NPs for photoacoustic imaging as well as photothermal therapy, as discussed below.

3. PHOTOACOUSTIC TOMOGRAPHY (PAT)

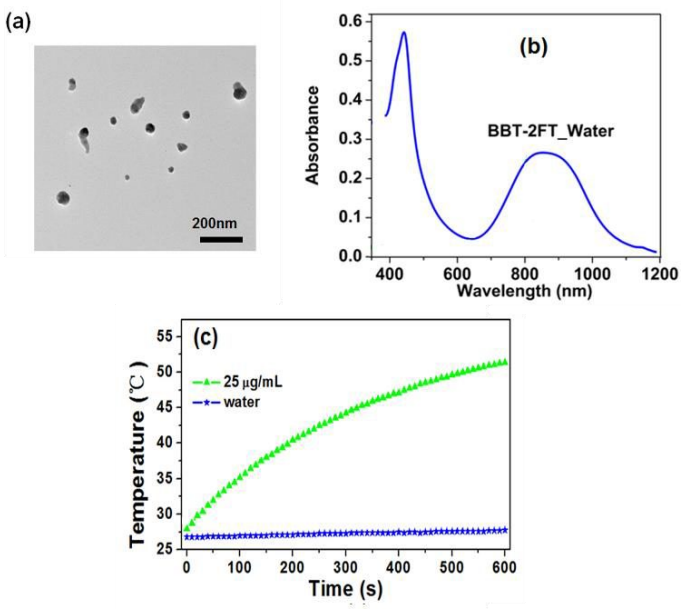

Fig. 2. (a) TEM image of BBT-2FT NPs, (b) Vis-NIR spectra of BBT-2FT NPs dispersed in water solution, (c) Temperature change plots of different concentrations of BBT-2FT NPs upon irradiation by an 808 nm laser with a power density of 1.77 W/cm^2 for 10 min.

Fig. 2b shows the UV-VIS-NIR extinction spectrum of the BBT-2FT NPs. It shows strong absorption in the NIR wavelength region which indicates the potential of BBT-2FT to act as a PAT contrast agent. We performed PAT experiments using our systems reported earlier.[30-32] To compare the PA signal from animal blood and BBT-2FT NPs, we performed experiments on animal blood/BBT-2FT NPs sample inside low density polyethylene (LDPE) tube (~0.59 mm inner diameter). The PA signal received by the 2.25 MHz UST was band pass filtered (1-10 MHz) and amplified with 50 dB gain. Finally, the signal was digitized by a DAQ card at 25 Ms/s and stored in computer. Fig. 3a shows the PA signals averaged 700 times from animal blood and BBT-2FT NPs (2 mg/mL). The signal from BBT-2FT NPs is ~10 times stronger than that from blood. We observed that the PA signal increased linearly with the concentration of BBT-2FT NPs. To check the feasibility of BBT-2FT NPs as a PAT contrast agent and determine their effective imaging depth at a wavelength of 803 nm, we acquired PA signals of BBT-2FT NPs embedded inside a chicken breast tissue. The LDPE tube filled with blood or BBT-2FT NPs (2 mg/mL) was embedded in the chicken breast tissue. PA signals were collected when the tube was placed at 1, 2, or 3 cm deep from the laser illuminated tissue surface. Fig. 3b shows the PA signals collected from BBT-2FT (2 mg/mL) at different depths by the 2.25 MHz UST. The BBT-2FT NPs were successfully detected in chicken breast tissue at depth of ~3.0 cm.

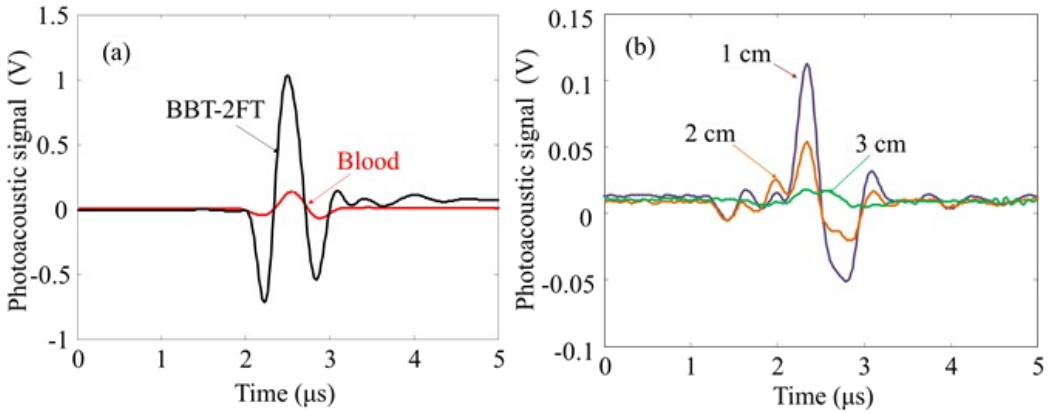

Fig. 3. Photoacoustic (PA) signal of BBT-2FT compared with blood. (a) PA signals of blood and BBT-2FT NPs (2 mg/mL) received by 2.25 MHz UST, (b) PA signals generated from BBT-2FT NPs in a LDPE tube embedded inside a chicken breast tissue at difference depths.

4. PHOTOTHERMAL THERAPY (PTT)

We investigate the *in vitro* photothermal therapy treatment of BBT-2FT NPs. HeLa cells (human cervical carcinoma cell lines) cultured in 12-well plates were incubated with 25 μg/mL BBT-2FT NPs solution for 6 h, after rinsing with PBS twice and being resupplied with fresh DMEM culture medium, then the cells were irradiated with an NIR laser (808 nm and 1.77 W/cm^2) for 10 min. Live/dead cells were differentiated by calcein AM (live cells, green fluorescence) and propidium iodide (PI) (dead cells, red fluorescence) co-staining after photothermal therapy treatment (Fig. 4). Almost 90% cells were killed after treatments of BBT-2FT NPs and laser irradiation. We have evaluated the photothermal cytotoxicity and biocompatibility of BBT-2FT. The tests showed that BBT-2FT NPs show minimal toxicity to HeLa cells without NIR laser irradiation. These results suggest that BBT-2FT NPs can serve as a promising photothermal agent for cancer therapy.

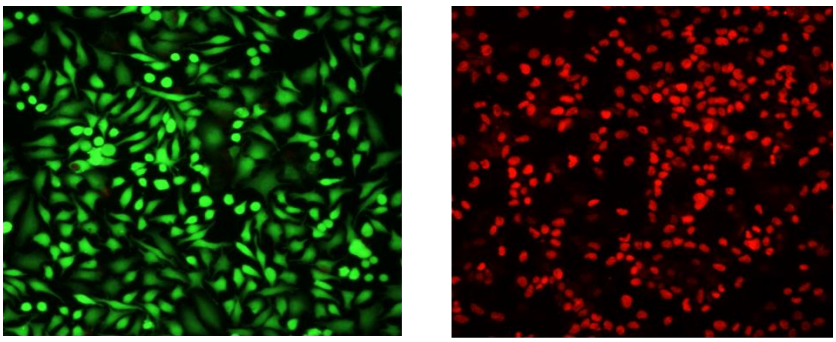

Fig. 4. Fluorescence images of calcein AM/PI co-stained HeLa cells after incubation for 6 h with BBT-2FT NPs (25 μg/mL) after being irradiated by laser (808 nm and 1.77 W/cm^2) for 10 min. (a) live cells without laser irradiation, (b) dead cells with laser irradiation.

5. CONCLUSIONS

In this work, we demonstrated a therapeutic agent based on BBT-2FT NPs for PAT and PTT. These NPs exhibit strong and broad NIR absorption, good colloidal stability, stronger PA signal than blood, higher photothermal conversion efficiency, and excellent photostability. The PA signal from BBT-2FT is ~10 times stronger than that from blood, and singles were from ~ 4 cm-deep inside tissue were recorded. And significant death of HeLa cells was observed due to the hyperthermal effect. These results demonstrate that the BBT–based NPs are promising theranostic agents for tumor imaging and therapy. In future we will investigate the potential of this contrast agent in vivo small animal study.

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