Temperature-Correlated Afterglow of a Semiconducting Polymer Nanococktail for Imaging-Guided Photothermal Therapy

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Abstract: Nanoparticles for photothermal therapy: Real-time temperature monitoring is critical to reduce the nonspecific damage during photothermal therapy (PTT); however, PTT agents that can emit temperature-related signals are rare and limited to few inorganic nanoparticles. We herein synthesize a semiconducting polymer nanococktail (SPNCT) that can not only convert photo-energy to heat but also emit temperature-correlated luminescence after cessation of light excitation. Such an afterglow luminescence of the SPNCT detects tumors more sensitively than fluorescence as a result of the elimination of tissue autofluorescence, while its temperature-dependent nature allows tumor temperature to be optically monitored under near-infrared (NIR) laser irradiation. Thus, SPNCT represents the first organic optical nanosystem that enables optical-imaging guided PTT without real-time light excitation.

Photothermal therapy (PTT) capitalizes on light-absorbing materials to generate hyperthermia under near-infrared (NIR) laser irradiation, provides new opportunities for cancer therapy.[1] However, the requirement of heating tumor tissue above 45°C or even higher for effective cancer ablation poses the potential risk to damage surrounding normal tissues.[2] To improve the therapeutic efficiency and reduce side effects in cancer therapy, temperature is a critical factor that needs to be precisely monitored during PTT.

One emerging strategy to monitor the temperature during PTT lies in the utilization of temperature-sensitive optical agents.[3] However, agents that can change their fluorescence intensity, spectral profile, or fluorescence lifetime in response to temperate are rare.[4] Until now, only PbS/CdS/ZnS quantum dots (QDs) and lanthanide-based upconversion nanoparticles have been revealed to have such ability for real-time monitoring of temperature during PTT.[5] However, both fluorescence and up-conversion luminescence require real-time light excitation, which not only interferes the photothermal irradiation light but also compromises the imaging sensitivity due to tissue autofluorescence. Moreover, organic nanoparticles with intrinsic heat-reporting ability have not been reported for real-time temperature monitoring during PTT.

Semiconducting polymer nanoparticles (SPNs) are a new class of organic photonic nanomaterials made from optically-active semiconducting polymers (SPs).[6] As SPNs are completely organic, they avoid the potential issue of metal-ion induced toxicity and have been shown to generally have good biocompatibility.[7] The structural versatility and excellent optical properties of SPNs have enabled a variety of imaging applications including fluorescence,[8] chemiluminescence,[9] photoacoustic[10] or photothermal control.[11] Recently, we found that poly(phenylenevinylene) (PPV) derivatives could continuously emit light even after removal of light excitation.[12] Such an afterglow luminescence avoids the tissue autofluorescence, offering a signal-to-background ratio (SBR, the mean luminescence intensity in the signal region divided by the mean luminescence intensity of the normal tissue background) that is significantly higher than fluorescence imaging.

In this study, we report the design and synthesis of semiconducting polymer nanococktail (SPNCT) with temperature-correlated afterglow luminescence for imaging-guided PTT. SPNCT comprises two major components (Figure 1): an amphiphilic poly(ethylene glycol) (PEG) grafted PPV (PPV-PEG) and a near-infrared (NIR) absorbing poly(silolodithiophene-alt-diketopyrrolopyrrole) (PCSD) serving as the temperature-monitoring sensor and photothermal agent, respectively. The hydrophobic PCSD polymer interacted with the hydrophobic backbone of PEG-PPV to form the nanoparticles with PEG chains covered on the surface. Such a multi-component structure is defined as nanococktail. The temperature sensitivity is an intrinsic characteristic of the afterglow from PPVs, because the photon-releasing decomposition of dioxetane units within PPV is thermodynamically controlled (Figure 1).[12] PCD within SPNCT has an electron-withdrawing structural unit (diketopyrrolopyrrole) and an electron-donating structural unit (silolodithiophene). Such a charge-transfer backbone favors nonradioactive deactivation and thus facilitates the generation of heat after light excitation. Afterglow-imaging guided PTT works as follows: after systemic administration of SPNCT, afterglow imaging enables more sensitive detection of tumor in living mice relative to fluorescence imaging; upon N IR light irradiation, SPNCT efficiently converts photon energy into heat, leading to increased temperature for PTT; during PTT, the temperature-correlated afterglow of SPNCT induced by light pre-irradiation allows to monitor the photothermal temperature. Such an afterglow imaging guided PTT also avoids the interference between light excitation for fluorescence and laser irradiation, facilitating real-time temperature monitoring for PTT.
To synthesize PPV with PEG grafts, monomers 1 and 2 were co-polymerized at the molar ratio of 1 to give PPV-Br (Figure 1a). Then, PPV-Br was reacted with sodium azide to substitute bromide with azide, affording the azide functionalized PPV. The resulted azide functionalized PPV was reacted with propargyl end-PEG to prepare PPV-PEG (Figure S2). The complete shift of the resonance peak of -CH$_2$Br was confirmed by proton nuclear magnetic resonance (1H NMR) spectroscopy (Figure S1, Supporting Information), which showed the peak at 7.51, 7.18, 5.35, 4.07, 3.95, 3.87, 1.44–1.27, and 1.04–0.75 ppm and the peak of the repeating unit of PEG (3.65 ppm) were detected by 1H NMR spectroscopy, confirming the structure of PPV-PEG (Figure S2, Supporting Information).

The nanococktail (SPN$_{CT}$) was synthesized via nano-coprecipitation of PPV-PEG, PCSD, and 1,2-distearoyl-sn-glycero-3-phosphethanolamine -poly(ethylene glycol)2000 (DSPE-PEG; Figure 1b,c). PCSD was chosen as the photothermal agent (Figure 1b), which had the strong absorption of NIR light, while DSPE-PEG was used to further stabilize the nanoparticles. The doping amount was optimized to be 150 w/w % (PCSD: PPV-PEG), wherein the nanoparticles had the highest amount of PCD but maintaining the stability (Figure S3, Supporting Information). Dynamic light scattering (DLS) showed that the SPN$_{CT}$ had a relatively narrow size distribution with an average hydrodynamic diameter of approximately 40 nm (Figure 1e), and transmission electron microscopy (TEM) revealed the spherical morphology of SPN$_{CT}$ (Figure 1e, inset). No precipitation and change in size were observed for SPN$_{CT}$ after storage for 30 days, demonstrating the excellent stability in aqueous medium (Figure S4, Supporting Information).

The optical and photothermal properties of SPN$_{CT}$ were studied in phosphate buffered saline (PBS; pH 7.4). SPN$_{CT}$ had two absorption peaks with the maxima at 509 and 770 nm, respectively (Figure 1f). The peak at 509 nm was contributed from PPV-PEG, which was used to induce afterglow luminescence after cessation of light irradiation. The peak at 770 nm was assigned to PCSD, which allowed NIR photothermal heating. The peak mass extinction coefficients of PPV and PCSD were 63 and 72 cm$^{-1}$mg$^{-1}$mL$^{-1}$, respectively. SPN$_{CT}$ exhibited a fluorescence spectrum ranging from 550 to 750 nm with an emission maximum at 600 nm. Under continuous laser irradiation at 808 nm, SPN$_{CT}$ showed strongly increased solution temperatures and reached plateau at $t = 360$ s (Figure 1g). The maximum photothermal temperature of SPN$_{CT}$ was 75°C and its photothermal conversion efficiency at 808 nm was calculated to be 35 %. The reversible heating-cooling operation showed that the maximum temperatures of SPN$_{CT}$ remained nearly the same for at least 5 cycles, proving the good photothermal stability (Figure S6).

To study photothermally enhanced afterglow, NIR laser and white-light irradiation were used to induce photothermal heating and afterglow of SPN$_{CT}$, respectively. The images were acquired immediately after NIR laser and white-light irradiation with the time gap of 2–3 s. With NIR laser
irradiation, the temperature of SPN\textsubscript{CT} solution was gradually increased (Figure 2a,b), resulting in increased afterglow intensity (Figure 2b). This was caused by the accelerated decomposition of the unstable PPV-dioxetane intermediate to PPV-aldehyde and photons.\(^{[12]}\) The afterglow intensity was increased by 3.0-fold upon photothermal heating of the solution from 30 to 55°C (Figure 2c). However, the fluorescence intensity remained the same regardless of the solution temperature (Figure 2b,d). A good linear correlation between temperature and afterglow signal of SPN\textsubscript{CT} was observed (Figure 2c,e), showing its potential to quantify the temperature and thus guide PTT. The afterglow of SPN\textsubscript{CT} decayed with a half-life of 4.5 min at room temperature (Figure S7). The afterglow of SPN\textsubscript{CT} could be repeatedly induced by white-light irradiation and no signal loss was observed under NIR laser multi-cycle heating. The optical spectra remained nearly the same, demonstrating the good photostability of SPN\textsubscript{CT} (Figure S8).

To examine PTT efficacy of SPN\textsubscript{CT} in vitro, 4T1 cells were incubated with SPN\textsubscript{CT} for 12 h and then irradiated for 5 min at 808 nm (0.5 W cm\(^{-2}\)). The cell status was qualitatively evaluated by calcein AM (live cells, green fluorescence) and propidium iodide (dead cells, red fluorescence) staining validated that SPN\textsubscript{CT} had the highest uptake in tumor validated that SPN\textsubscript{CT} had the highest uptake in tumor (Figure S9) and also quantitatively measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assays (Figure S10). SPN\textsubscript{CT} had no obvious cytotoxicity in the absence of laser irradiation (Figure S9,S10), demonstrating the good cytocompatibility. In contrast, almost all the cells were ablated by SPN\textsubscript{CT} after laser irradiation, indicating the high PTT efficacy of SPN\textsubscript{CT}.

The ability of SPN\textsubscript{CT} for afterglow imaging guided PTT was tested on xenograft 4T1 tumor mouse model. The temperature, afterglow luminescence, and fluorescence signals were first acquired in real time after intratumoral injection of SPN\textsubscript{CT}. In accordance with the in vitro data, a good linear correlation between temperature and afterglow signal in tumor region was detected for SPN\textsubscript{CT}, demonstrating the feasibility of afterglow imaging guided PTT in vivo (Figure S11). The afterglow luminescence of SPN\textsubscript{CT} was then tested and compared with fluorescence for passively targeted imaging of tumor in living mice. Afterglow and fluorescence signals were longitudinally acquired in real time after systemic administration of SPN\textsubscript{CT} through tail-vein injection. Both signals gradually increased over time and reached peak at 24 h post-injection, but the SBR of afterglow images was higher than that of fluorescence images at each time point (Figure 3a,b). At \(t = 24\) h, the SBR of afterglow images was 154 ± 10, 7.3-fold higher than the fluorescence images (21 ± 3 (Figure 3b)). The significantly higher SBR for afterglow imaging was attributed to the eliminated tissue autofluorescence. Such a high SBR of afterglow allowed to clearly distinguish the tumor signal from the background noise at \(t = 1\) h post-injection; in contrast, fluorescence imaging only could do so at \(t = 24\) h post-injection. These results coincided with the ex vivo and in vivo tissue-penetration studies, showing the significantly higher tissue penetration and imaging sensitivity of afterglow over fluorescence (Figure S12). The ex vivo biodistribution data further validated that SPN\textsubscript{CT} had the highest accumulation in tumor followed by liver, intestine, stomach, and other major organs (Figure S13). Such a favorable accumulation in tumor was consistent with our previous observation, which should be due to the small size and stable nanostructure of semiconducting polymer brush based nanoparticles.\(^{[13]}\)

According to the afterglow and fluorescence imaging results, SPN\textsubscript{CT} had the highest accumulation in tumor at 24 h post-injection, and thus PTT was conducted at this time point. The 4T1-tumor bearing mice were irradiated at 808 nm (0.3 W cm\(^{-2}\)) for 6 min. The tumor temperature for SPN\textsubscript{CT} was measured by infrared thermal imaging (Figure S17). The afterglow luminescence images were acquired for 10 s after irradiation by white light (0.1 W cm\(^{-2}\)) for 1 min. Afterglow and fluorescence images were acquired for 0.1 s at 600 ± 10 nm upon excitation at 500 ± 10 nm.
was observed during PTT (Figure 3e). Therefore, this data confirmed that SPN CT could not only convert photo energy into heat for PTT, but also emit temperature-dependent afterglow signals for temperature monitoring during PTT. Moreover, owing to the higher tissue penetration and imaging sensitivity of the afterglow luminescence of SPN CT, it had the potential to monitor the temperature in deep tissue (Figure S17, S18).[14]

To further evaluate the antitumor effect for SPNCT-based afterglow imaging guided PTT, tumor sizes for different treatment groups were continuously monitored every other day for 17 days after PTT. The tumor growth for SPNCT-treated mice were successfully inhibited after laser irradiation, which was not for other control groups (saline-treated mice with or without laser irradiation, and SPNCT-treated mice without laser irradiation; Figure 4a and Figure S15). Note that the irradiation dose was generally lower than the previous studies using other organic and inorganic nanoparticles (Table S1). No significant weight loss of mice was observed throughout the whole experimental period (Figure 4b), and no noticeable histopathological damage was observed in livers, kidneys, and spleens for all groups after PTT (Figure 4c and Figure S16), indicating the biosafety of SPNCT. In contrast, typical nucleus dissociation (Figure 4c) and a large fraction of apoptotic cells (green fluorescence; Figure 4d) were clearly detected for the tumor tissues of SPNCT-treated mice after PTT. These data verified that SPNCT-mediated PTT had a high and specific antitumor effect.

In summary, we have synthesized optically active SPs and made them into nanococktails for afterglow-imaging guided PTT. Such an organic nanoagents (SPNCT) had a high photothermal conversion efficiency (35%), and emitted afterglow luminescence after cessation of light excitation. With a small size (40 nm) and PEGylated surface, SPNCT passively targeted tumor in living mice, showing a highest accumulation in tumor rather than in liver. Owing to

Figure 3. In vivo tumor imaging and afterglow-guided PTT. a) Fluorescence and afterglow luminescence images of 4T1 tumor-bearing living mice at different times after intravenous injection of SPNCT (300 µg mL\(^{-1}\) based on the concentration of PCSD, 200 µL). b) SBRs for fluorescence and afterglow imaging of tumor in living mice treated with SPNCT as a function of post-injection time. c) Thermal and afterglow images of tumor in living mice at 24 h after intravenous injection of SPNCT under laser irradiation at 808 nm for different times with a power of 0.3 W cm\(^{-2}\). d) Mean tumor temperature during laser irradiation after intravenous injection of saline or SPNCT into 4T1 tumor-bearing living mice at post injection time of 24 h. Error bars are based on standard error of mean (SEM; \(n=4\)). e) Quantification of in vivo afterglow luminescence of tumor as a function of photothermal temperature. Afterglow luminescence images were acquired for 10 s with an open filter after irradiation with white light (0.1 W cm\(^{-2}\)) for 1 min. Fluorescence images were acquired for 0.1 s at 600 ± 10 nm upon excitation at 500 ± 10 nm.

Figure 4. Evaluation of anti-tumor efficacy for afterglow-guided PTT. a) Tumor growth curves and b) body weight data of mice after intravenous injection of saline or SPNCT with or without laser irradiation at 808 nm (\(n=4\)). Error bars indicated standard error of mean (SEM; \(n=4\)). c) H&E staining for tumors of mice treated with saline or SPNCT with or without laser irradiation. d) Immunofluorescent staining of caspase-3 for tumors of mice treated with saline or SPNCT with or without laser irradiation. Green fluorescence indicates staining of caspase-3, while blue fluorescence indicates nucleus staining. All the treatments were performed 24 h after intravenous injection of saline or SPNCT. The tumors for H&E staining and immunofluorescent staining of caspase-3 were collected after 17 days of treatment.
the eliminated tissue autofluorescence, the afterglow of SPNCT enabled delineation of tumor in a more sensitive and faster way than fluorescence. Moreover, because the afterglow intensity had a good linear correlation with temperature, it allowed the photothermal temperature of tumor to be monitored under NIR laser irradiation. As a result of such ideal biophysical and photothermal properties along with its completely benign organic composition, SPNCT permitted effective eradication of tumors with a minimal damage to normal tissues in living mice. Thus, this study reports the first organic nanosystem that enables optical imaging guided PTT without real-time light excitation.

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Conflict of interest

The authors declare no conflict of interest.

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Afterglow, a cocktail: A semiconducting polymer nanococktail (SPN_{CT}) with temperature-correlated afterglow luminescence is designed for imaging-guided photothermal therapy. The afterglow intensity of the SPN_{CT} has a good linear correlation with temperature, allowing the photothermal temperature of the tumor to be monitored under NIR laser irradiation.