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Near-infrared Absorbing Amphiphilic Semiconducting Polymers for Photoacoustic Imaging

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Development of photoacoustic (PA) imaging agents is crucial to advancing PA imaging in biology and medicine. In this study, we report the design and synthesis of near-infrared (NIR) absorbing amphiphilic semiconducting polymers that can spontaneously self-assemble into homogenous water-soluble nanoparticles for PA imaging of tumor in living mice.

Photoacoustic (PA) imaging is a hybrid technique that irradiates the tissue with a pulsed laser and measures optically induced ultrasound signals.¹ Compared with traditional optical imaging techniques, PA imaging provides deeper tissue penetration with higher spatial resolution, because ultrasound scatters much less relative to light when passing through tissue. To image the biological process or activity of biomarkers, small-molecule organic dyes,² fluorescent proteins,³ porphyrins,⁴ inorganic nanoparticles,⁵ carbon nanomaterials⁶ and two-dimensional materials⁷ have been studied as exogenous contrast agents for molecular PA imaging. However, these materials have limitations, such as photo-bleaching for organic dyes, difficulty to be bio-metabolized or ion-induced toxicity for inorganic nanoparticles and broad PA profiles for carbon and two-dimensional materials.⁸ Thus, development of alternating PA imaging agents is essential.

Semiconducting polymer nanoparticles (SPNs) made from opto-electronically active semiconducting polymers (SPs) have formed as a new class of optical agents due to their excellent optical properties and good biocompatibility.⁹ SPNs have been applied for luminescence imaging applications such as cell tracking,¹⁰ tumor imaging,¹¹ ultrafast hemodynamic imaging,¹² drug-induced injury¹³ and neuroinflammation.¹⁴ In addition, SPNs can efficiently convert light energy into heat, permitting sensitive PA imaging and efficient photothermal therapy of tumors.¹⁵ We have developed SPNs into PA imaging agents for

a board range of applications including imaging of tumor,¹⁶ lymph nodes,⁹ pH,¹⁷ protein sulfenic acids¹⁸ and reactive oxygen species (ROS).¹⁹

In terms of chemistry, SPNs are generally prepared *via* mini-emulsion or nanoprecipitation. To endow SPNs with good biodistribution, amphiphilic copolymers are required during nanoparticle formation.^{11,14} Thus, most SPNs can be considered as micellar nanoparticles, which could potentially encounter dissociation and subsequently cause aggregation during blood circulation.²⁰

To avoid the potential dissociation issue of SPNs, we herein report the design and synthesis of two near-infrared (NIR) absorbing amphiphilic SPs that can self-assemble into nanoparticles for PA imaging (Figure 1a). These amphiphilic polymers contain diketopyrrolopyrrole (DPP)-based semiconducting backbone grafted with hydrophilic poly (ethylene

Figure 1. Synthesis and characterization of amphiphilic SP nanoparticles. (a) Schematic illustration of synthesis of amphiphilic SP nanoparticles. (b) Chemical structures of SPs: PCD-PEG and PFD-PEG. (c) Photos of nanoparticle solutions (30 µg mL⁻¹). (d) TEM image of PCD-PEG nanoparticles. (e) DLS of PCD-PEG and PFD-PEG. (f) Cell viability of 4T1 cells after incubation with PCD-PEG or PFD-PEG solutions at various concentrations.

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glycol) (PEG) *via* click reaction. In the following, we first describe their synthesis, followed by analysis of their optical, PA and photothermal properties. At last, the proof-of concept application is demonstrated for *in vivo* tumor imaging.

Results and Discussion

The amphiphilic SPs, PEG-grafted poly(cyclopentadithiophene-*alt*-diketopyrrolopyrrole) (PCD-PEG) and PEG-grafted poly(fluorene-*alt*-diketopyrrolopyrrole) (PFD-PEG), were synthesized via a graft-on approach (Scheme 1). Monomer 1 was synthesized by 2,6-dibromo-4H-cyclopenta-[2,1-*b*:3,4-*b'*]dithiophene and 1,6-dibromohexane under basic conditions (Figure S1, Supplementary Information). Monomer 1 was polymerized with monomer 2 to yield PCD-Br *via* Pd-catalyzed Stille coupling reaction, while monomer 3 was polymerized with monomer 4 to yield PFD-Br *via* Pd-catalyzed Suzuki polymerization. Then, PCD-Br and PFD-Br were respectively reacted with sodium azide to obtain PCD-N₃ and PFD-N₃. The ¹H NMR of PCD-N₃ showed that the proton resonances of -CH₂-N₃ was changed from 3.36 of -CH₂-Br to 3.21 (Figure S2, Supplementary Information), implying that the bromide group was completely substituted by azide group. The ¹H NMR of PFD-N₃ (Figure S3, Supplementary Information) also proved this. At last, PCD-N₃ and PFD-N₃ were respectively react with methoxy-PEG-alkyne (Mn = 2000) through copper(I)-

Scheme 1. Synthetic routes of PCD-PEG and PFD-PEG. Reagents and conditions: i) 1,6-dibromohexane, tetrabutylammoniumiodide (TBAI), NaOH (50% aq), 75 °C, 45 min; ii) bis(triphenylphosphine) palladium(II) dichloride (PdCl₂(PPh₃)₂), 2,6-di-tert-butylphenol, toluene, 100 °C, 2 h; iii) palladium tetrakis(triphenylphosphine) (Pd(PPh₃)₄), potassium carbonate (K₂CO₃), methyltriocetyl ammonium chloride, toluene/H₂O, 100 °C, 2 h; iv) NaN₃, tetrahydrofuran (THF)/N,N-dimethylformamide (DMF), 25 °C, overnight; v) copper(I) bromide (CuBr), N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA), methoxy-PEG-alkyne, THF, 25 °C, 48 h.

Figure 2. Optical and photothermal characterization of PCD-PEG and PFD-PEG. (a) UV-visible-NIR absorption spectra of PCD-PEG and PFD-PEG. (b) Photothermal temperatures of PCD-PEG and PFD-PEG solutions as a function of laser irradiating time. (c) Stability study of PCD-PEG and PFD-PEG under photothermal heating and cooling cycles. (d) Thermal images of PCD-PEG and PFD-PEG at their respective maximum temperatures. The concentrations of the optical components of both SPs were 18 $\mu\text{g mL}^{-1}$ in 1x PBS (pH = 7.4). The laser irradiation wavelength was at 808 nm with a power of 1 W cm^{-2} .

catalyzed alkyne-azide cycloaddition (CAAC) reaction to obtain the final amphiphilic polymers: PCD-PEG and PFD-PEG. As shown in the ¹H NMR of PCD-PEG (Figure S4, Supplementary Information), the major resonance peak of PEG was found at 3.6 ppm and the peaks of SP backbone was found at 7.87-6.68, 4.27-4.12, 1.45-1.13 and 0.87 ppm. These data demonstrated the successful synthesis of the amphiphilic SPs.

Both amphiphilic SPs could be dispersed very well in phosphate buffer solution (PBS) (Figure 1c). Transmission electron microscopy (TEM) (Figure 1d and Figure S5 in Supplementary Information) showed that PCD-PEG and PFD-PEG had uniform spherical morphology with the diameters of ~40 and 30 nm, respectively. Dynamic light scattering (DLS) (Figure 1e) showed the average hydrodynamic diameters of PCD-PEG and PFD-PEG were 39 and 29 nm, respectively. No obvious change in size was observed for both polymers after storage in PBS (pH = 7.4) even for 30 days (Figure S6, Supplementary Information). In addition to this, PCD-PEG showed high stability during storage in fetal bovine serum (FBS) for 10 days (Figure S7, Supplementary Information). The zeta potentials of PCD-PEG and PFD-PEG were measured to be -4.29 and -2.82 mV, respectively. Furthermore, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) assay showed that both SPs were non-cytotoxic to 4T1 cells (Figure 1f). These results indicated that these SP nanoparticles had ideal aqueous stability and cytocompatibility for biological applications.

The absorption and photothermal properties of PCD-PEG and PFD-PEG were tested in PBS solution. At the same mass concentration of the optical components, the maximum absorption peak of PFD-PEG and PCD-PEG were at 675 and 803 nm, respectively (Figure 2a). The red-shifted absorption of PCD-PEG relative to that of PFD-PEG was attributed to the stronger electron-donating ability of cyclopentadithiophene as compared with that of fluorene. Under continuous laser irradiations at 808 nm, both SPs showed gradually increased

solution temperatures and reached plateau at $t = 360$ s (Figure 2b). The maximum photothermal temperature for PCD-PEG was 86°C , which was ~ 2.7 -fold higher than that for PFD-PEG (32°C) (Figures 2b & 2d). Calculated by the natural cooling curve, the photothermal conversion efficiency of PCD-PEG at 808 nm was 37% , ~ 6 -fold higher than that of PFD-PEG (6%). To study the photothermal stability, both PCD-PEG and PFD-PEG solutions were under reversibly heating and natural cooling for 5 times (Figure 2c), their maximum temperature remained nearly the same. Thus, these data showed that PCD-PEG was a better photothermal agent as compared to PFD-PEG, probably because of the stronger electron-delocalization of the PCD backbone.

The PA properties of PCD-PEG and PFD-PEG were also studied in PBS. The PA spectra of both polymers were close to their absorption, showing the maximum intensity at 680 nm and 790 nm for PFD-PEG and PCD-PEG, respectively. At the same concentration of optical components, the PA amplitude of PCD-PEG at 790 nm was 1.69-fold higher than PFD-PEG at 680 nm (Figure 3a). In addition, the PA amplitude of PCD-PEG was 5.34-fold higher than the gold nanorods (GNRs) with the absorption maximum close to 790 nm (Figure 3b). The PA amplitudes and images of PCD-PEG at 790 nm were determined at a series of concentrations of the optical components from 5 to $80\ \mu\text{g mL}^{-1}$ (Figures 3c&3d), showing a good linear relationship between the PA signals and the polymer concentrations (Figure 3c). These PA data were consistent with the photothermal results, further proving that PCD-PEG was a better agent to convert light energy into heat.

Because PCD-PEG had the longer and brighter NIR PA signals than PFD-PEG, it was further evaluated for PA imaging of tumor in living mice. For the control mice, only weak PA signals at 790 nm can be detected in the tumor areas because of the low absorption of hemoglobin molecules in the NIR region (Figure 4a). On the contrary, the PA signals in the tumor areas gradually increased over time after systemic administration of PCD-PEG through intravenous injection (Figure 4b). At $t = 2$ h post-injection, the PA signals in the tumor areas was ~ 2 -fold higher than the background signals, indicating that PCD-PEG quickly

same mass concentration of the optical components. (c) The PA amplitudes as a function of concentrations of PFD-PEG (680 nm) or PCD-PEG (790 nm). (d) PA images of PCD-PEG solutions at different concentrations. The error bars represent the standard deviations of five separate measurements.

accumulated into the tumor site. The maximum PA signals in the tumor areas were found at $t = 24$ h post-injection, which was ~ 7 -fold higher than that of the background signals. At such a time point, the maximum intensity projection (MIP) and 3D PA images obviously illustrated that PA signals came from the areas both within and outside the blood vessels of the tumor. Thereby, these images and quantified signals showed that PCD-PEG not only passively targeted tumor but also partially extravasated from tumor vasculatures possibly due to their relatively small diameter (~ 40 nm).

Figure 4. *In vivo* imaging of PCD-PEG. (a) Representative PA maximum intensity projection (MIP) (upper) and 3D (lower) images of tumor of a living mouse 0, 8, 24 and 32 h post-injection of PCD-PEG ($30\ \mu\text{g mouse}^{-1}$) *via* tail vein. (b) PA intensities of tumors in PCD-PEG-injected living mice ($n = 3$) as a function of post-injection time. (c) *In vivo* real-time PA spectra extracted from the tumors of living mice after systemic administration of PCD-PEG or saline for 24 h.

To further confirm the nanoparticle accumulation in tumor, the real-time *in vivo* PA spectra were extracted from the tumor areas of PCD-PEG-injected mice and plotted in Figure 4c. The spectrum of PCD-PEG-injected mice clearly differed from that of saline-injected mice, but resembled that of PCD-PEG in aqueous solution (Figures 4c & 3a). Thus, these data showed the enhancement of PA signals was induced by the accumulation of PCD-PEG in the tumor of living mice. The *ex vivo* biodistribution at $t = 32$ h post-injection showed PCD-PEG was mainly accumulated in spleen, tumor and liver (Figure S8, Supplementary Information). Note that tumor had a relatively strong PA signals which was even higher than that of liver. Our previous reports showed that the SPNs prepared with coprecipitation with amphiphilic PEG-based polymers usually had the highest uptake in liver instead of tumor despite the similar PEG coating.^{11,16} Thereby, the ideal biodistribution of PCD-PEG should be attributed to its non-dissociable nanostructure passivated by a dense PEG shell that minimized the reticuloendothelial uptake and promoted the EPR effect. In

Figure 3. *In vitro* PA properties of PCD-PEG, PFD-PEG and GNR. (a) PA spectra of PCD-PEG and PFD-PEG in PBS ($\text{pH} = 7.4$). (b) PA signals of PCD-PEG and GNR at 790 nm at the

conjunction with its high PA brightness, PCD-PEG could delineate the tumor of living mice in a clearer way (tumor signal-to-background = 7) as compared with the previously-reported SPNs (tumor signal-to-background $\approx$ 2 to 5).¹⁶

Conclusions

We have designed and synthesized two NIR-absorbing amphiphilic SPs composed of a hydrophobic SP backbone grafted by the hydrophilic PEG chains and compared their photothermal and PA properties. With stronger charge transfer backbone, PCD-PEG had $\sim$ 6-fold higher photothermal conversion efficiency (37 %) and 1.69-fold higher PA brightness as compared with PFD-PEG. Moreover, the PA signals of PCD-PEG at 790 nm was 5.34-fold higher than the GNRs with the similar absorption maximum. In association with self-assembled small nanoparticulate structure (40 nm), the strong PA brightness of PCD-PEG allowed it to image the tumor of living mice with the tumor signal-to-background as high as 7. By virtue of low liver uptake, PCD-PEG holds great promise for other PA imaging tasks. Thus, this study provides a new generation of purely organic nanoagents prepared *via* self-assembly approach for PA imaging.

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References

- a) V. Ntziachristos, D. Razansky, *Chem. Rev.*, 2010, **110**, 2783; b) L. V. Wang, S. Hu, *Science* 2012, **335**, 1458; c) Q. Miao, K. Pu, *Bioconjugat. Chem.*, 2016, **27**, 2808.
- a) Q. Fan, K. Cheng, Z. Yang, R. Zhang, M. Yang, X. Hu, X. Ma, L. Bu, X. Lu, X. Xiong, W. Huang, H. Zhao, Z. Cheng, *Adv. Mater.* 2015, **27**, 843. b) J. Zhang, X. Zhen, P. K. Upputuri, M. Pramanik, P. Chen, K. Pu, *Adv. Mater.*, 2017, DOI: 10.1002/adma.201604764.
- a) R. M. Hoffman, *Nat. Rev. Cancer*. 2005, **5**, 796; b) G. S. Filonov, A. Krumholz, J. Xia, J. Yao, L. V. Wang, V. V. Verkhusha, *Angew. Chem. Int. Ed.*, 2012, **51**, 1448.
- a) J. F. Lovell, C. S. Jin, E. Huynh, H. Jin, C. Kim, J. L. Rubinstein, W. C. Chan, W. Cao, L. V. Wang, G. Zheng, *Nat. Mater.* 2011, **10**, 324. b) Y. Zhang, M. Jeon, L. J. Rich, H. Hong, J. Geng, Y. Zhang, S. Shi, T. E. Barnhart, P. Alexandridis, J. D. Huizinga, M. Seshadri, W. Cai, C. Kim, J. F. Lovell, *Nat. Nanotechnol.* 2014, **9**, 631.
- a) J. V. Jokerst, A. J. Cole, D. Van de Sompel, S. S. Gambhir, *ACS Nano*, 2012, **6**, 10366; b) J. Liu, X. Zheng, L. Yan, L. Zhou, G. Tian, W. Yin, L. Wang, Y. Liu, Z. Hu, Z. Gu, C. Chen, Y. Zhao, *ACS Nano*, 2015, **9**, 696.
- a) A. De La Zerda, C. Zavaleta, S. Keren, S. Vaithilingam, S. Bodapati, Z. Liu, J. Levi, B. R. Smith, T. J. Ma, O. Oralkan, Z. Cheng, X. Y. Chen, H. J. Dai, B. T. Khuri-Yakub, S. S. Gambhir, *Nat. Nanotechnol.* 2008, **3**, 557; b) J. Liu, C. Wang, X. Wang, X. Wang, L. Cheng, Y. Li, Z. Liu, *Adv. Funct. Mater.* 2015, **25**, 384.
- a) K. Yang, L. Feng, X. Shi, Z. Liu, *Chem. Soc. Rev.*, 2013, **42**, 530; b) J. Lin, X. Chen, P. Huang, *Adv. Drug Delivery Rev.* 2016, **105**, 242.
- a) P. Huang, J. Lin, X. Wang, Z. Wang, C. Zhang, M. He, K. Wang, F. Chen, Z. Li, G. Shen, D. Cui, X. Chen, *Adv. Mater.* 2012, **24**, 5104; b) C. S. Jin, J. F. Lovell, J. Chen, G. Zheng, *ACS Nano* 2013, **7**, 2541.
- a) K. Pu, A. J. Shuhendler, J. V. Jokerst, J. Mei, S. S. Gambhir, Z. Bao, J. Rao, *Nat. Nanotechnol.* 2014, **9**, 233; b) K. Sun, Y. Tang, Q. Li, S. Yin, W. Qin, J. Yu, D. T. Chiu, Y. Liu, Z. Yuan, X. Zhang, C. Wu, *ACS Nano* 2016, **10**, 6769; c) K. Pu, N. Chattopadhyay, J. Rao, *J. Controlled Release*, 2016, **240**, 312.
- K. Pu, A. J. Shuhendler, M. P. Valta, L. Cui, M. Saar, D. M. Peehl, J. Rao, *Adv. Healthcare Mater.*, 2014, **3**, 1292.
- a) C. Wu, S. J. Hansen, Q. Hou, J. Yu, M. Zeigler, Y. Jin, D. R. Burnham, J. D. McNeill, J. M. Olson, D. T. Chiu, *Angew. Chem. Int. Ed.*, 2011, **50**, 3430; b) Y. Lyu, Y. Fang, Q. Miao, X. Zhen, D. Ding, K. Pu, *ACS Nano*, 2016, **10**, 4472; c) H. Zhu, Y. Fang, X. Zhen, N. Wei, Y. Gao, K. Q. Luo, C. Xu, H. Duan, D. Ding, P. Chen, K. Pu*, *Chemical Science*, 2016, **7**, 5118–5125.
- G. Hong, Y. Zou, A. L. Antaris, S. Diao, D. Wu, K. Cheng, X. Zhang, C. Chen, B. Liu, Y. He, J. Z. Wu, J. Yuan, B. Zhang, Z. Tao, C. Fukunaga, H. Dai, *Nat. Commun.* 2014, **5**, 4206.
- A. J. Shuhendler, K. Pu, L. Cui, J. P. Uetrecht, J. Rao, *Nat. Biotechnol.*, 2014, **32**, 373.
- a) X. Zhen, C. Zhang, C. Xie, Q. Miao, K. L. Lim, K. Pu, *ACS Nano*, 2016, **10**, 6400; b) C. Xie, P. K. Upputuri, X. Zhen, M. Pramanik, K. Pu*, *Biomaterials*, 2017, **119**, 1–8.
- Y. Lyu, C. Xie, S. A. Chechetka, E. Miyako, K. Pu, *J. Am. Chem. Soc.*, 2016, **138**, 9049.
- a) K. Pu, J. Mei, J. V. Jokerst, G. Hong, A. L. Antaris, N. Chattopadhyay, A. J. Shuhendler, T. Kurosawa, Y. Zhou, S. S. Gambhir, Z. Bao, J. Rao, *Adv. Mater.*, 2015, **27**, 5184. b) C. Xie, P. K. Upputuri, X. Zhen, M. Pramanik, K. Pu, *Biomaterials*, 2017, **119**, 1–8.
- Q. Miao, Y. Lyu, D. Ding, K. Pu, *Adv. Mater.* 2016, **28**, 3662.
- Y. Lyu, X. Zhen, Y. Miao, K. Pu, *ACS Nano*, 2017, **11**, 358–367.
- K. Pu, A. J. Shuhendler, J. Rao, *Angew. Chem. Int. Ed.*, 2013, **52**, 10325;
- C. J. Rijcken, C. J. Snel, R. M. Schifferers, C. F. van Nostrum, W. E. Hennink, *Biomaterials*, 2007, **28**, 5581.