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COMMUNICATION

Dendronized Semiconducting Polymer as Photothermal Nanocarrier for Remote Activation of Gene Expression

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Abstract: Regulation of transgene systems is imperative to develop innovative medicines. However, noninvasively remote control of gene expression has been rarely developed and remains to be challenging. We herein synthesize a near-infrared (NIR) absorbing dendronized semiconducting polymer (DSP) and utilize it as a photothermal nanocarrier not only to efficiently deliver genes but also to spatiotemporally control gene expression in conjunction with heat-inducible promoter. DSP has a high photothermal conversion efficiency (44.2%) at 808 nm, permitting fast transduction of NIR light into thermal signals for intracellular activation of transcription. Such a DSP-mediated remote activation can rapidly and safely result in 25- and 4.5-fold increments in the expression levels of proteins in living cells and mice, respectively. This study thus provides a promising approach to optically regulate transgene systems, which has the potential to be translated into nanomedicines for on-demand therapeutic transgene dosing.

Regulation of cellular behaviors and transgene systems can provide useful tools to understand their physiological roles and potentially lead to innovative therapies. In this regard, gene therapy that utilizes viral or non-viral vectors to deliver exogenous nucleic acids into specific cells has become a promising therapeutic approach to treat inherited genetic diseases and cancers.[1] However, it is challenging to precisely regulate gene expression at designated location and time so as to minimize off-target expression.[2] Encoding tissue-specific promoters in therapeutic nucleic acids is one feasible solution,[3] which however is less likely to be generalized for various diseases. Recently, gas molecules,[4] radio waves[5] and light[6] have been used to remotely switch on gene expression. Among them, light is the most ideal inducer due to its low toxicity, easy tunability and high spatiotemporal resolution. However, current methods are generally limited to ultraviolet[7] and visible light[6a,b] with shallow tissue penetration, preventing them from *in vivo* applications; moreover, optical responsive components are knocked in through synthetic biology to trigger the complex cascade response,[6b,c] partially complicating their implementation.

Semiconducting polymer nanoparticles (SPNs) have evolved as versatile optical agents for molecular imaging.[8] As SPNs comprise completely benign organic components, they bypass the issue of metal ion induced toxicity but possess optical advantages equal or sometimes superior to inorganic semiconductor nanoparticles.[9] SPNs have also been revealed to efficiently convert photon energy into heat, making them applicable for photoacoustic imaging and photothermal therapy.[10] Particularly, they often exhibit higher absorption and photothermal conversion efficiencies as compared with other nanoparticles such as carbon nanotubes and gold nanorods, enabling faster heating.[11] Based on such excellent photothermal properties, we recently developed SPN-based bioconjugates that specifically targeted and rapidly activated the protein thermosensitive ion channels in neurons.[11a] These studies imply that SPNs could serve as photothermal nanomodulators to optically regulate cellular behaviors.[12]

We herein report the development of a near-infrared (NIR) absorbing dendronized semiconducting polymer (DSP) and demonstrate its proof-of-concept application as a photothermal nanocarrier and intracellular nanotransducer for remote activation of gene expression. DSP comprises three key components (Figure 1a): the hydrophobic semiconducting backbone, the cationic third-generation polyamidoamine (PAMAM3) side chains, and the neural poly(ethylene glycol) (PEG) blocks, which serve as the NIR photothermal nanotransducer, the gene vector and the water-solubility enhancer, respectively. The conversion of photothermal signal generated from DSP into the cue for gene expression is realized by simple incorporation of plasmids with the gene of interest at the downstream of heat shock promoter (HSP70). Thus, gene expression can be regulated by HSP70 and activated by photothermal signals. The working mechanism starts with the electrostatic attraction induced formation of nanocomplexes between DSP and HSP70-regulated gene (Figure 1b). After cellular internalization, the gene is released from the complex and enters into cellular nucleus, while DSP retains in cytoplasm. Upon laser irradiation at 808 nm, DPS generates heat, stimulating the transformation of heat shock factor (HSF) from monomers to trimers; HSF trimers then translocate to the cellular nucleus, bind to heat shock element (HSE) in the HSP70 and activate transcription.[13] As such, DSP sequentially acts as the

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gene nanocarrier and the intracellular photothermal nanotransducer to efficiently deliver and remotely control transgene expression. Note that although carbon nanotubes have been used to activate HSP70 in the previous studies, they are simply used as the heater and gene delivery need to be conducted in a separate step.[14]

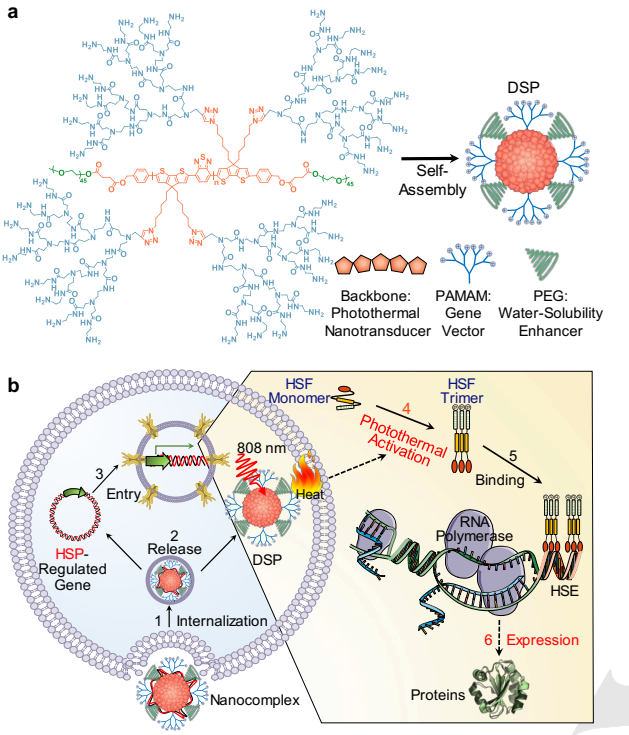

Figure 1. Design DSP as photothermal nanocarrier and nanotransducer for remote activation of gene expression. (a) Chemical structure and self-assembly of DSP. (b) Illustration of DSP-mediated gene delivery and remote photothermal activation of gene expression under NIR laser irradiation at 808 nm.

DSP was synthesized *via* a graft-on approach (Schemes S1 and S2, Supporting Information). Pd-catalyzed Suzuki polymerization of PEG-functionalized endcapping agent (4), 4,4'-bis-(5-bromopentyl)-4H-cyclopenta[2,1-b:3,4-b']dithiophene (5), and 2,1,3- benzothiadiazole-4,7-bis (boronic acid pinacol ester) (6) led to the PEG-endcapped triblock copolymer (P1). The bromide groups of P1 were converted into the azide groups, allowing it to undergo the copper(I)-catalyzed alkyne-azide cycloaddition (CAAC) reaction to graft PAMAM2.5 onto it. At last, the resulted grafted SP (P3) reacted with ethylenediamine *via* aza-Michael addition reaction to convert PAMAM2.5 into the third-generation form (PAMAM3), affording the final polymer (DSP). The intermediate polymers and DSP were characterized by NMR (Figures S1-S3, Supporting Information). DSP could be directly dissolved in aqueous solution and buffer, making it feasible for gene delivery.

DSP had a broad absorption peak in the NIR region ranging from 500 to 900 nm with a maximum at 635 nm, and its fluorescence maximum was located at 791 nm (Figure 2a). Such a NIR optical feature is beneficial for *in vivo* applications, enabling utilization of NIR light excitation to generate both fluorescence and photothermal signals. Upon continuous laser irradiation at 808 nm, the temperature of DSP solution gradually increased and reached plateau of 58.8ºC at t= 480 s (Figure 2b). The photothermal conversion efficacy of DSP was calculated to be 44.2±2.8%, which was higher than gold nanorods (~17%).[11a] Moreover, the maximum temperature of DSP could be adjusted using different laser powers and it remained nearly unchanged after 6 reversible heating and natural cooling cycles, proving its excellent photothermal stability (Figure S4, Supporting Information).

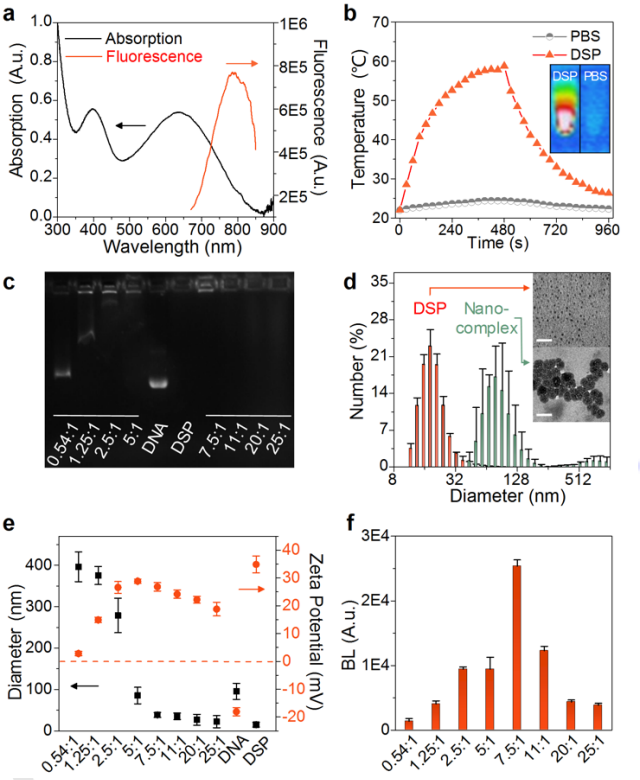

Figure 2. *In vitro* characterization of DSP and its transfection capability. (a) Absorption and fluorescence spectra of DSP in 1×PBS at pH 7.4. (b) Solution temperatures of DSP (132 μg/mL) as a function of laser irradiating time. The laser wavelength was 808 nm and its power intensity was 0.67 W cm^{-2}. Inset: thermal images of DSP and PBS solutions at their respective maximum temperatures. (c) Agarose gel electrophoresis of DNA, DSP and DSP/DNA nanocomplexes at various N/P ratios. (d) DLS profiles of DSP and DSP/DNA nanocomplex with the N/P ratio of 5:1. Inset: representative TEM images of DSP and DSP/DNA complex; scale bars indicate 100 nm. (e) Hydrodynamic diameters and zeta potential of DNA, DSP and DSP/DNA nanocomplexes at various N/P ratios. (f) Transfection efficacy of DSP/DNA nanocomplexes at various N/P ratios. Error bars represented standard deviations of three separate measurements.

To evaluate the complexation of DSP with genes, the pSV40-*Luc* plasmid (luciferase as the reporter protein) was used as an example, and the gel retardation, diameters, zeta potentials of the DSP/plasmid nanocomplexes were studied at different N/P ratios. Agar gel electrophoresis revealed that plasmids were fully loaded by DSP at a low N/P ratio of 5:1, because nearly all the plasmids were retarded in the slots and no migration band was observed (Figure 2c). Dynamic light scattering (DLS) and transmission electron microscopy (TEM) were conducted to analyze the condensation between DSP and plasmids. DSP itself had a spherical morphology with the average hydrodynamic diameter of ~15 nm (Figure 2d and Figure S5, Supporting Information), remaining nearly unchanged after storage for 42 days (Figure S6, Supporting Information). After complexation with plasmids, the morphology remained spherical. However, the diameters dramatically decreased from 396 to 38 nm with increasing the N/P ratio from 0.54:1 to 7.5:1 (Figure 2e). This was well observed for non-viral vectors,[15] and could be contributed to enhanced DSP/plasmid condensation with increased N/P ratios. The diameters slightly changed when the N/P ratio was larger than 7.5:1, indicating the formation of compact and stable nanocomplexes. The zeta potential of the nanocomplexes increased from 3 to 29 mV with increasing the N/P ratio and reached maximum at the N/P ratio of 5:1 (Figure 2e). These data proved that DSP was an ideal carrier with the efficient gene loading and condensation capabilities.

The ability of DSP for *in vitro* gene transfection was studies in Hela cells using luciferase genes (pSV40-*Luc*) as the reporter gene. The transfection efficacy was evaluated by the expression

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level of luciferase, which can be quantified with bioluminescence (BL). With increased N/P ratio, the BL intensity gradually increased, and reached maximum at the N/P ratio of 7.5:1 (Figure 2f). This N/P ratio was consistent well with the gene loading and condensation data, wherein the compact and stable DSP/plasmid nanocomplexes formed. When the N/P ratios were above 7.5:1, the BL intensity slightly decreased, probably caused by the over condensation that blocked plasmid release. In addition to the excellent performance in transfection, DSP showed good cytocompatibility even at the high concentration (Figure S7, Supporting Information).

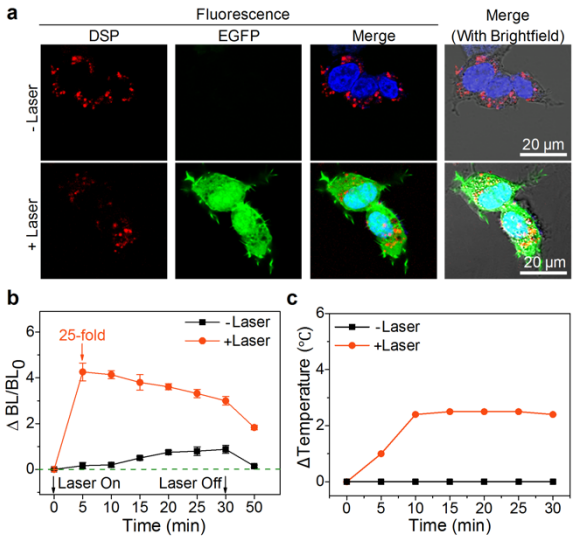

Figure 3. *In vitro* DSP-mediated photothermal activation of gene expression. (a) Fluorescence images of HeLa cells transfected with DSP/pHSP70-*EGFP* nanocomplexes with the N/P ratio of 7.5:1 with and without laser irradiation at 808 nm (0.75 W cm^{-2}) for 30 min. Quantification of changes in the BL intensities (b) and temperature (c) of HeLa cells transfected with DSP/pHSP70-*Luc* nanocomplexes with the N/P ratio of 7.5:1 with and without laser irradiation at 808 nm (0.75 W cm^{-2}) for 30 min. The excitation and emission wavelengths were 640/655-710 nm for DSP, 488/500-550 nm for EGFP and 405/410-470 nm for Hoechst. Error bars represented standard deviations of three separate measurements.

To test the photothermal ability of DSP to remotely control transgene expression, enhanced green florescence proteins (EGFPs) and luciferases were employed as the reporter proteins at the downstream of HSP70, and activation was conducted on HeLa cells. The sequences were designed according to the previous literature.[16] Hela cells were incubated with the DSP/pHSP70-*EGFP* plasmid nanocomplexes at the optimal N/P ratio of 7.5:1, recovered with fresh media and treated with or without laser irradiation at 808 nm for 30 min. With the NIR fluorescence of DSP, confocal fluorescence microscopy could be applied to track the location of DSP and the expression of EGFP in cells. After incubation, the NIR fluorescence from DSP was observed in the cytoplasm for both laser treated or non-treated cells, indicating the efficient cellular internalization of DSP/pHSP70-*EGFP* plasmid nanocomplexes (Figure 3a). The location of DSP was also ideal to trigger HSP70-regulated gene, as associated HSF monomers located in cytoplasm.[11] Only upon laser irradiation, green fluorescence was observed, showing the remotely controlled expression of EGFP. Such a laser-triggered expression was not observed in the absence of HSP70 (Figure S8, Supporting Information), confirming that the activation was triggered by DSP-mediated photothermal transduction.

To quantify the DSP-mediated photothermal activation of gene expression, similar cell experiments were conducted on HeLa cells using the DSP/pHSP70-*Luc* plasmid nanocomplexes at the optimal N/P ratio of 7.5:1. The luciferase expression level was longitudinally monitored for ~70, 000 cells and quantitatively described as the incremental percentage of BL intensities ($\Delta BL/BL_0$) (Figure 3b). Meanwhile, the solution temperature was monitored along with the laser irradiation (Figure 3c). The highest expression appeared after laser irradiation for only 5 min. At this time point, $\Delta BL/BL_0$ was 25-fold higher than that without irradiation, indicating the significantly enhanced gene expression level triggered by DSP in a rapid manner. Note that during the laser irradiation, the solution temperature was increased by ~2.4 ºC at most. Such a temperature increase did not cause obvious cytotoxicity (Figure S9, Supporting Information). These cell data verified that DSP served as the cytocompatible photothermal nanocarrier and nanotransducer to remotely control gene expression. Note that light irradiation of cellular medium alone could trigger transgene expression due to the heat generation from water absorption.[17] However, due to the weak photothermal effect of water, it requires high power and long irradiation time, which may cause cytotoxicity issue.

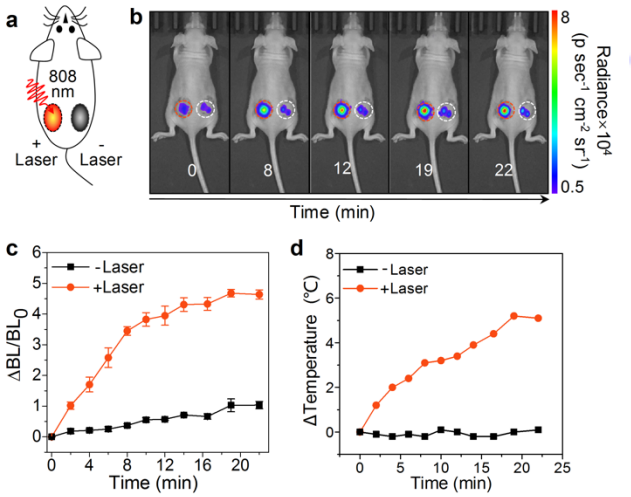

Figure 4. *In vivo* DSP-mediated photothermal activation of gene expression. (a) Illustration of laser irradiation in living nude mice. (b) whole-animal BL images at representative time-points with (left) and without (right) laser irradiation at 808 nm (0.42 W cm^{-2}). The red/white dashed circles indicated the respective regions subcutaneously implanted with the HeLa cells pellets (2, 520,000 cells) transfected with DSP/pHSP70-*Luc* nanocomplexes and treated with/without laser irradiation. Quantification of changes in the BL intensities (c) and temperature (d) as a function of time with (left) and without (right) laser irradiation. Error bars represented standard deviations of three separate measurements.

To validate the potential of DSP for *in vivo* activation of gene expression, the HeLa cells pellets (~2, 520,000 cells) transfected with DSP/pHSP70-*Luc* nanocomplexes were subcutaneously injected into the back of living mice (Figure 4a). The BL images were longitudinally recorded and quantified for the areas with or without laser irradiation. To minimize the temperature increase, the laser treatment was set in a discontinuous manner (Figure S10, Supporting Information). BL intensity gradually increased along with laser irradiation and reached maximum at 19 min (Figures 4b&4c), while it slightly changed without laser irradiation. Such a phenomenon was not observed for the cells transfected with luciferase gene at the downstream of the constitutive promoter (SV40) (Figure S11, Supporting Information). This proved that the expression of luciferase was selectively controlled by the photothermal generation of DSP under high tissue penetrating NIR light irradiation at 808 nm. At t =19 min, $\Delta BL/BL_0$ was 4.5-fold higher than that without laser irradiation. Note that the temperature increased only by 5.2 ºC at most during laser irradiation (Figure 4d). The highest temperature was 39.5 ºC below the threshold temperature to induce apoptosis (43 ºC) (Figure S12, Supporting Information). These *in vivo* data clearly validate the feasibility of using DSP for remotely controlled gene expression at desired location and time in living animals.

In conclusion, we have synthesized a multifunctional water-soluble polymer (DSP) that can not only efficiently deliver genes into cells but also convert NIR light into heat for photothermal applications. In association with heat inducible promoter

regulated genes, DSP acts as the intracellular photothermal nanotransducer to remotely activate gene expression upon NIR light irradiation at 808 nm. Real-time monitoring of luminescence indicates that gene expression can be rapidly triggered and significantly enhanced by 25- and 4.5-fold within minutes in living cells and mice, respectively. Thus, this study provides an innovative polymeric approach to noninvasively control gene expression at designated location and time. To the best of our knowledge, this is the first polymer system that integrates the functionalities of gene delivery and photothermal activation. In view of its completely benign organic components and NIR absorption with high-tissue penetration, such kind of polymers holds promise to be developed into nanomedicines for controlled therapeutic transgene dosing.

Acknowledgements

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Keywords: dendronized semiconducting polymer • non-viral gene carrier • photothermal effect • light activation • nanoparticles

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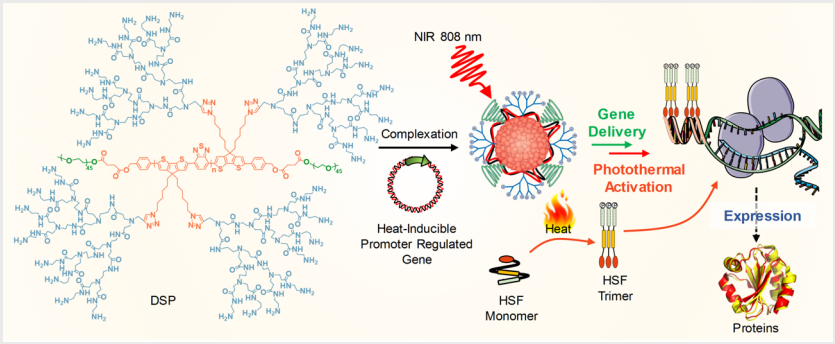

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Title: Dendronized Semiconducting Polymer as Photothermal Nanocarrier for Remote Activation of Gene Expression

TOC Description

Remote Control: A near-infrared (NIR) absorbing dendronized semiconducting polymer (DSP) is synthesized and utilized as a photothermal nanocarrier not only to efficiently deliver gene but also to photothermally control gene expression at designated location and time.