Harnessing Croconaine Organic Photosensitizers for a Milder Surface-Mediated Transfection

Ferdinandus[a#], *Wen Ling Lenice Tan*[a#], *Jie Ren Tan*[a], *Hiang Kwee Lee*[a], *Qiang Zhu*[b,c], *Enyi Ye*[b,c], *and Chi-Lik Ken Lee*[a,c]*

a. Ferdinandus, Wen Ling Lenice Tan, Jie Ren Tan, Hiang Kwee Lee, and Chi-Lik Ken Lee.
School of Chemistry, Chemical Engineering and Biotechnology, Nanyang Technological University. 21 Nanyang Link, Singapore 637371.

b. Qiang Zhu and Enyi Ye.
Institute of Materials Research and Engineering (IMRE), Agency for Science, Technology, and Research (A*STAR). 2 Fusionopolis Way, Innovis, Singapore 138634.

c. Qiang Zhu, Enyi Ye, and Chi-Lik Ken Lee.
Institute of Sustainability for Chemicals, Energy and Environment ($ISCE^2$), Agency for Science, Technology, and Research (A*STAR). 1 Pesek Road, Singapore 627833.

E-mail: ken_lee@isce2.a-star.edu.sg

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Abstract

Polydopamine-based materials, notably Polydopamine-polyethyleneimine (PDA-PEI), have gained considerable interest for surface-mediated transfection. However, the high laser power density required to achieve high transfection efficiency poses a significant challenge in preserving cell viability. An organic photosensitizer CRO32TMI was developed to improve the photothermal conversion capability of PDA-PEI. The modified PDA-PEI-CRO32TMI exhibited remarkable photothermal and photostability properties upon NIR irradiation, enabling it to achieve better transfection efficiency at lower laser power density as compared to the traditional solution or lipid-based transfection methods.

1. Introduction

Gene delivery shows great potential as a therapy for a range of health conditions, including cancer and inherited diseases.[1,2] In traditional gene delivery methods, viral vectors and transfection reagents like the lipid-based Lipofectamine 2000 are used to facilitate the transfer of DNA and RNA.[2] However, the mutagenic potential of viral vectors and the challenges of achieving high transfection efficiency through solution-mediated transfection due to numerous extracellular barriers, have led to a demand for a more effective approach to delivering genetic materials.[2,3] Surface-mediated transfection approach has garnered significant interest in recent times owing to its potential to facilitate the direct interaction between DNA/RNAs and cells by immobilizing genetic material on its surface. More importantly, this method offers a promising solution to overcome the membrane barrier by utilizing photothermal materials that can generate mild hyperthermia (43-45°C).[4] As a result of this mild hyperthermia, the cell membrane is disrupted by the creation of transient pores, which allow the genetic material immobilized on the surface to penetrate directly into the cell.[4,5] This novel approach results in a more localized and amplified gene expression, thereby increasing the efficiency of transfection in comparison to conventional transfection methods.

Polydopamine-based materials have gained popularity as surface-mediated transfection surfaces due to their adhesive properties and unique photothermal conversion capabilities.[6,7] When coupled with a near-infrared region (NIR, 808 nm) laser, they can generate transient pores. Zhang et al. demonstrated that Polydopamine-polyethyleneimine (PDA-PEI) could be easily produced through a co-deposition method via Michael Addition **(Figure 1)**.[5] PDA-PEI demonstrated excellent photothermal heating efficiency, leading to increased transfection efficacy in Human umbilical vein endothelial cells (HUVECs), which are notoriously difficult to transfect.[5,7] However, it is worth noting that high laser power density (808 nm, > 2 W cm^{-2}) was often necessary to achieve an efficient gene transfection with PDA-based materials, which can potentially harm cells and tissues.[6] Therefore, it is imperative to explore alternative photothermal materials with higher efficacy to enable milder transfection conditions.

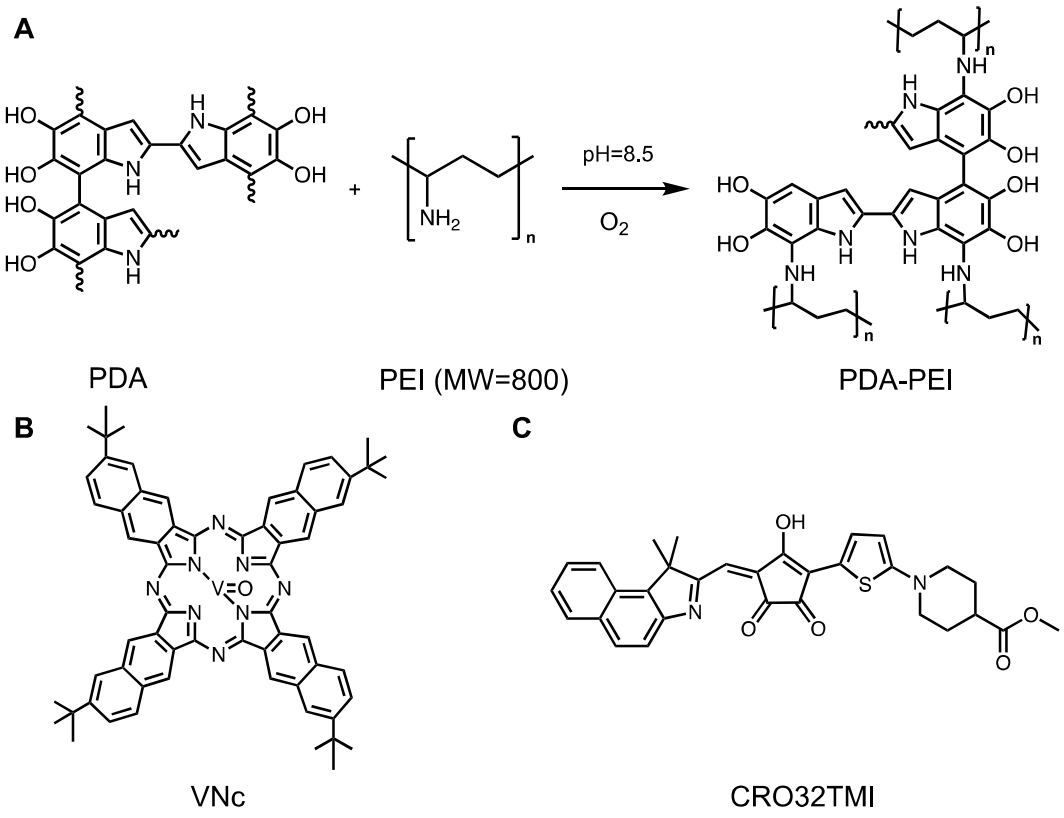

Figure 1. PDA-PEI polymer and added photothermal agents. (A) Schematic illustration of PDA-PEI synthesis by Michael Addition.[8] (B) Vanadyl 2,11,20,29-tetra-tert-butyl-2,3-naphthalocyanine. (C) CRO32TMI.

The modification of PDA-PEI with different photothermal agents (PTAs) was proposed to enhance its heating efficiency for milder transfection conditions and transfection efficiency for harder-to-transfect cell lines. Gold nanorods (AuNP) and organometallic dyes, such as Vanadyl 2,11,20,29-tetra-tert-butyl-2,3-naphthalocyanine (VNc) (Figure 1B), are typical photothermal agents with optimal absorption in the NIR region (808 nm) that have demonstrated high photothermal conversion efficiency and exceptional photostability, making them ideal candidates for modification.[8,9] However, the use of metal-based photothermal dyes raises a concern regarding their long-term cytotoxicity. Thus metal-free organic photosensitizers are mostly the choice for consideration in designing a transfection platform with high photothermal conversion efficiency while maintaining good photostability for milder transfection conditions.[10]

Croconaine (CRO) is a type of organic photosensitizer featuring a donor-acceptor-donor (D-A-D) structure that provides extended π-conjugation. These dyes typically display robust

absorption in the NIR region, excellent photostability (**Figure 2**), and low cytotoxicity.[11] As a result, CRO dyes represent an ideal photothermal agent for the modification. A CRO dye, CRO32TMI, was synthesized via a two-step condensation reaction and incorporated into PDA-PEI **(Scheme S1)**. Herein, we report the utilization of CRO32TMI to enhance the photothermal effect of PDA-PEI, while simultaneously maintaining excellent photostability, thereby facilitating milder transfection conditions and achieving high transfection efficiency as compared to the traditional lipid-based transfection.

2. Results and Discussion

In this study, the photothermal conversion efficiency (PCE) of the different PTAs was first evaluated. CRO32TMI exhibited an impressive PCE of 37.5%, while V-Nc only showed 8.2% and AuNP showed 30.4%. Next, the photothermal properties of PDA-PEI-CRO32TMI under 808 nm laser irradiation were monitored using an infrared (IR) thermal camera in aqueous conditions that simulate transfection conditions. At a laser power density of 0.84 W cm^{-2}, PDA-PEI-CRO32TMI induced a similar increase in surface temperature (21.6°C) as compared to PDA-PEI-VNc (21.4°C) within 2 minutes of irradiation **(Figure 2A)**. This temperature increase was higher than that of unmodified PDA-PEI (8.4°C) and PDA-PEI-AuNP (14.4°C). The increase in surface temperature achieved by PDA-PEI-CRO32TMI was sufficient to achieve the desired mild photothermal effect for up to 30 minutes of laser irradiation. The photoswitchability and stability of PDA-PEI-CRO32TMI were also investigated in the dry state through three sequential irradiation cycles and the consistency of the surface temperature achieved after each cycle indicated the stable photothermal properties of PDA-PEI-CRO32TMI (**Figure 2B**). Overall, the photophysical characteristics suggested that PDA-PEI-CRO32TMI could lead to improved heating efficacy with good photostability for milder surface-mediated transfection.

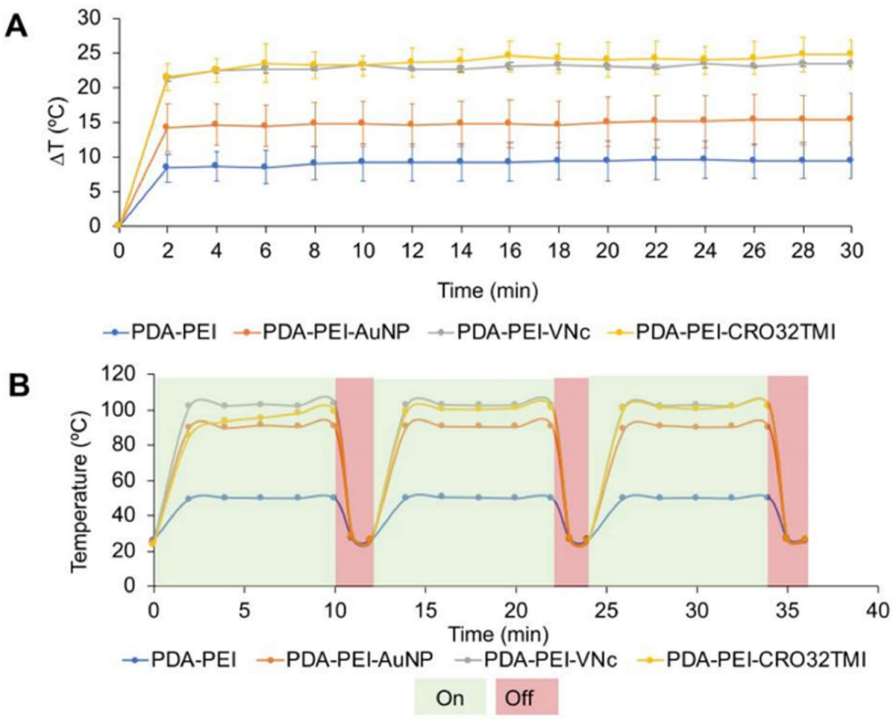

Figure 2. Photophysical properties of various PDA-PEI polymer mixtures. (A) Photothermal effect and (B) Photostability under 808 nm (0.84 W cm^{-2}, 30 mins) irradiation.

HeLa, Hep-G2, and C2C12 cell lines were subjected to traditional solution-mediated gene delivery using Lipofectamine 2000 and photothermal surface-mediated transfection **(Figure 3)**. The observed mean fluorescence intensity (MFI) of the green fluorescence protein (GFP) expression utilizing the liposome-based Lipofectamine 200 was relatively low.[12, 13] In comparison, the use of surface-mediated transfection enhanced the MFI of Hep-G2 and C2C12 across all the PDA-PEI surfaces. Meanwhile, HeLa displayed a significant increase of MFI only in the PTA-modified PDA-PEI surfaces **(Figure 4)**. Together, these results indicated that the surface-mediated transfection mechanism using PDA-PEI allows for a significant improvement of transfection efficiency in Hep-G2 and C2C12 as compared to Lipofectamine 2000. Further modification of the PDA-PEI surfaces using PTAs could boost the transfection efficiency higher owing to the elevated surface temperature allowing for surface-mediated transfection.[4]

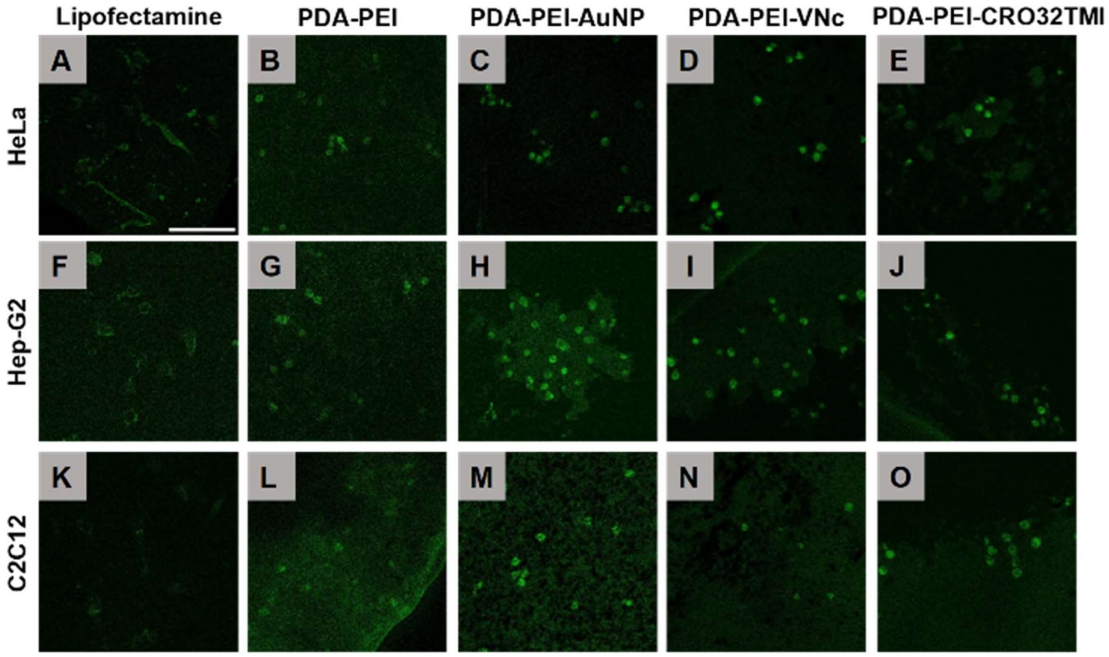

Figure 3. Confocal images of surface-mediated transfection with PDA-PEI and its various modified surfaces in comparison to solution-mediated transfection with Lipofectamine 2000: (A–E) HeLa cells, (F–J) Hep-G2 cells, and (K–O) C2C12 cells. Scale bar = 100 µm.

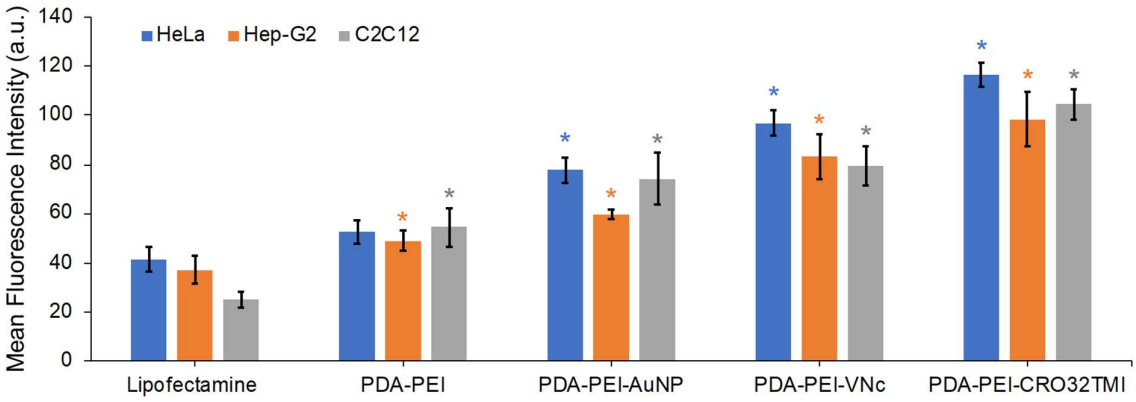

Figure 4. Mean fluorescence intensity (MFI) obtained for HeLa, Hep-G2 and C2C12 in solution-mediated transfection, surface-mediated transfection with PDA-PEI, and its various modified surfaces. Student's T-test *p<0.05 compared to solution-mediated transfection. Experiments were repeated three times independently.

The transfection efficiency of the surface-mediated polymer was compared across the three cell lines using various PDA-PEI polymers embedded with the different PTAs and the brightest MFI was observed with PDA-PEI-CRO32TMI, indicating the highest transfection

efficiency across all three cell lines. All three cell lines grown on the PDA-PEI-CRO32TMI displayed a significant increase of MFI compared to those on the PDA-PEI-AuNP. However, only C2C12 cells grown on the PDA-PEI-CRO32TMI displayed a significant MFI increase as contrasted to the cells grown on PDA-PEI-VNc **(Figure 5)**. This suggests that CRO32TMI is capable to increase transfection efficiency compared to AuNP due to its higher heating efficacy. Nonetheless, CRO32TMI performed equally tantamount to VNc owing to their similar photothermal profile (**Figure 2**). Despite this, PDA-PEI-CRO32TMI showed a promising prospect for enhancing transfection efficiency in the difficult-to-transfect C2C12 cell line as it is a widely used cell line with low transfection efficiency with Lipofectamine reagents.[16]

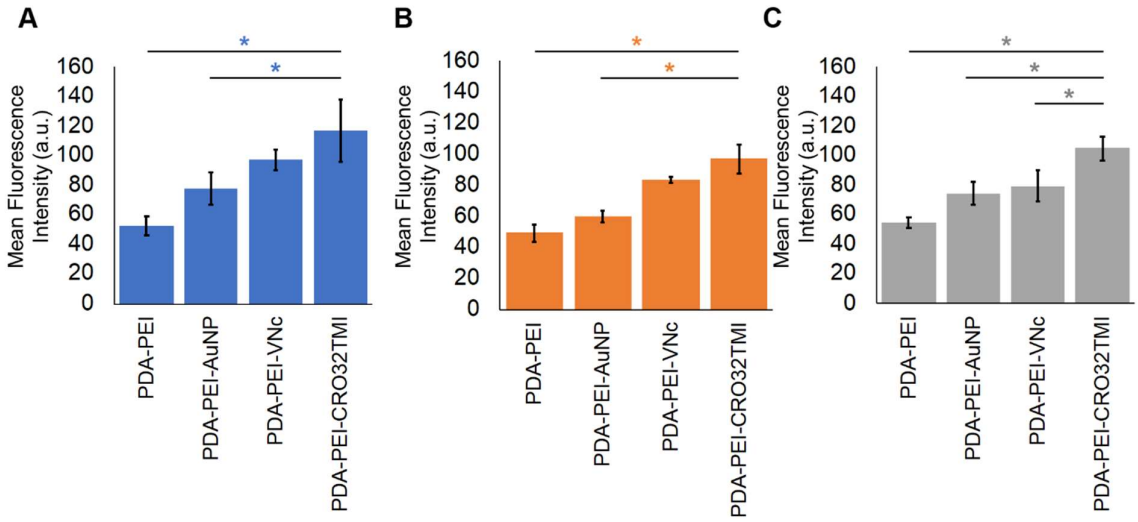

Figure 5. Mean fluorescence intensity (MFI) obtained for (A) HeLa, (B) Hep-G2 and (C) C2C12 by surface-mediated transfection. Student's T-test *p<0.05. Experiments were repeated three times independently.

3. Conclusion

To conclude, an organic photosensitizer CRO32TMI was synthesized and incorporated into PDA-PEI. PDA-PEI-CRO32TMI exhibited excellent photothermal and photostability properties, demonstrating its potential as a highly efficient and durable transfection enhancer. Successful demonstration of this modification has led to an increase in transfection efficiency in Hep-G2 and C2C12 cell lines. Compared to commercial gold nanorods, transfection efficiency significantly improved and was similar when using a metallic-based organic PTA VNc. Thus, modification of the PDA-PEI complex with an organic photosensitizer CRO32TMI offers a breakthrough strategy which holds enormous promise for surface-

mediated gene delivery with lower laser power density and has significant implications for future research in this field.

Supporting Information

Supporting Information is available upon request from the authors.

Acknowledgements

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[#] Ferdinandus and Wen Ling Lenice Tan contributed equally to this work.

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