Red-Light-Photosensitized NO Release and Its Monitoring in Cancer Cells with Biodegradable Polymeric Nanoparticles

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Nitric Oxide (NO) is a small, free radical involved in the regulation of several physiological and pathophysiological processes [1] including cardiovascular diseases, bacterial infections [2] and cancer [3]. This multifaceted role has stimulated, over the last few years, a massive interest for the development of unconventional therapeutic approaches based on the NO use to tackle important diseases. However, the strict dependence of the biological effects of NO on its concentration [3] requires the generation of the radical with precise spatiotemporal control. Light represents a powerful tool to fulfil this need in a minimally invasive way and with high accuracy by using appropriate photoprecursors namely NO Photodonors (NOPDs) [4]. The development of organic NOPDs activatable in the so-called “therapeutic window” with highly biocompatible and tissue-penetrating red light is desirable and challenging [5].

In this contribution, we demonstrate that one-photon red-light excitation of Verteporfin, a clinically approved photosensitizer (PS) for photodynamic therapy, triggers NO release from a blue-light activatable NOPD (NBFNO) with an improvement of about 300 nm toward longer and more biocompatible wavelengths [6]. NO photorelease is photosensitized by the lowest triplet state of the PS, more likely by a catalytic photoinduced electron transfer. In view of biological applications, the water-insoluble PS and NOPD have been co-entrapped within water-dispersible and biocompatible polymeric nanoparticles (NPs) of mPEG-PCL without affecting the NO release process. Moreover, the spectroscopic prerequisites and the restricted environment of the NPs permit the green-fluorescent co-product (NBF) formed concomitantly to NO photorelease to communicate with the PS via Förster resonance energy transfer (FRET). This results in an enhancement of the typical red emission of the PS offering the possibility of a dual colour optical reporter useful for the real-time monitoring of the NO release through fluorescence techniques (figure 1).

Biological tests were performed using different types of cancer cell lines to ascertain the validity of this strategy applied to the polymeric NPs as potential nanotherapeutics. The results of cell viability experiments and fluorescence investigation in cells demonstrate the occurrence of the NO release under red-light illumination also in the biological environment. This confirms that the adopted strategy provides a valuable tool for generating NO from an already available NOPD, otherwise activatable with the poorly biocompatible blue light, without requiring any chemical modification and the use of sophisticated irradiation sources and opens intriguing prospects in biomedical research for studies where precise and spatiotemporally controlled concentrations of NO are required.

References

[1] L. J. Ignarro, Arch. Pharm. Res., 32, (2009), 1099-1101.
[2] R. Devine, M. Douglass, M. Ashcraft, N. Tayag, H. Handa, ACS Appl. Mater. Interfaces, 13, (2021), 19613−19624.
[3] D. Fukumura, S. Kashiwagi, R.K. Jain, Nat. Rev. Cancer, 6, (2006), 521−534.
[4] S. Sortino, J. Mater. Chem., 22, (2012), 301-318.
[5] R. Weissleder, Nat. Biotechnol. 19, (2001), 316−317.
[6] A. Fraix, C. Parisi, G. Longobardi, C. Conte, A. Pastore, M. Stornaiuolo, A. C. E. Graziano, M. E. Alberto, A. Francés-Monerris, F. Quaglia, S. Sortino, Biomacromolecules, 24, (2023) , 3887–3897.

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Figures

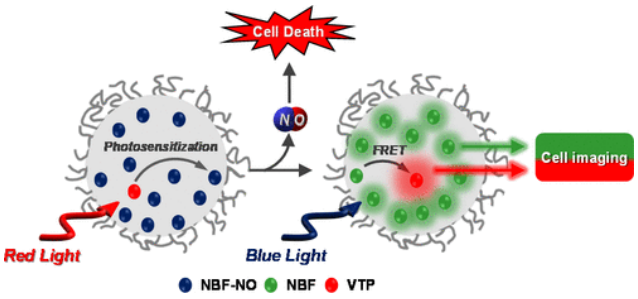

Figure 1. Schematic for the photosensitized NO release and its monitoring.