doi:10.1246/cl.2010.1008
Published on the web August 14, 2010

Editor's Choice

PEGylated Polymer Micelle-based Nitric Oxide (NO) Photodonor with NO-mediated Antitumor Activity

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PEGylated polymer micelles containing 4-nitro-3-trifluoromethylphenyl units within the core moiety were prepared, and their phototriggered nitric oxide (NO)-generating ability was confirmed by electron spin resonance (ESR) spin-trapping and the Griess method. These micelles were found to be capable of delivering exogenous NO into tumor cells in a photocontrolled manner and showed an NO-mediated antitumor effect, indicating the usability of this molecular system in NO-based tumor therapy.

Nitric oxide (NO) is a free radical endogenously synthesized in the body and plays multiple physiological roles, including vasodilation, angiogenesis, neurotransmission, and immune response.[1] Depending on its concentration and localization, NO has diverse biological functions.[2] For example, NO can both promote and inhibit tumor progression depending on its local concentration. Relatively high concentrations of NO have been shown to inhibit tumor progression by promoting apoptosis.[2] This means that a molecular system capable of site-specific delivery of a high amount of NO should be useful in antitumor treatment. Due to the poor bioavailability of NO, great efforts have been made to develop a methodology for the controlled delivery of exogenous NO in living systems.[3] One promising approach is the exploitation of a photochemical process which can be spatiotemporally controlled by manipulating the incident light. Until now, various NO-photogenerative compounds (NO photodonors) have been developed and applied for the examination of photocontrolled NO delivery.[4,5] However, the development of NO photodonors with excellent water solubility, biocompatibility, and tumor specificity is still challenging. In this regard, we newly designed a PEGylated polymer micelle-based NO photodonor which contains NO photogenerative units in the core moiety. It is well recognized that PEGylated polymer micelles several tens of nanometers in size are potent carriers of various therapeutics agents.[6] They show high colloidal stability under physiological conditions and preferentially accumulate in solid tumors by the enhanced permeation and retention (EPR) effect.[7] Here, we wish to communicate the preparation of a PEGylated polymer micelle-based NO photodonor and its potential for NO-based therapeutic application.

The PEG-based amphiphilic block copolymer used in this study [PEG-*b*-PNTP (PEG: 5000 g mol^{-1}, PNTP: 3600 g mol^{-1}, $DP_{PNTP}(n) = 11$] in Figure 1) was synthesized via conjugation between PEG-*b*-poly(4-chloromethylstyrene)[8] and 4-nitro-3-trifluoromethylphenol (NTP) in the presence of potassium carbonate.[9] Nitrobenzene derivatives having bulky substituents at the *ortho*-position are known to generate NO upon appropriate light irradiation though a nitro-to-nitrite photorearrangement followed by the rupture of the O–NO bond (Figure 1).[5] Micellization of PEG-*b*-PNTP was carried out by dialysis. The hydrodynamic diameter and polydispersity index (PDI) of the resulting micelles (ND micelles) were determined by dynamic light scattering to be 42.3 nm and 0.15, respectively (Figure S2).[9] The low PDI value indicates the formation of uniform micelles without any secondary aggregates. In addition, the ND micelles showed a neutral surface charge (ζ potential: 0.03 mV in 10 mM phosphate buffer, pH 7.4) and remained dispersed without any change in size for at least one month. It should be mentioned that the hydrodynamic diameter of the ND micelles is suitable for tumor-specific accumulation via the EPR effect (i.e., below 100 nm).[7]

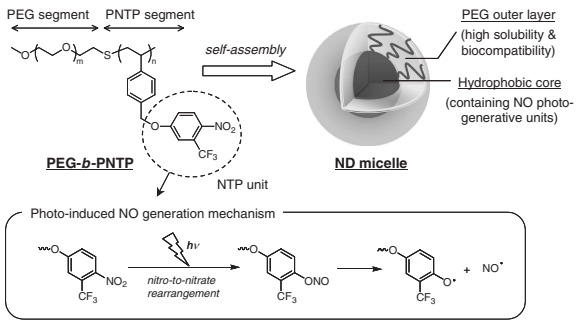

Figure 1. Schematic illustration of the PEGylated polymer micelle-based NO photodonor (ND micelles) used in this study.

Phototriggered NO generation from the ND micelles was first tested by electron spin resonance (ESR) spin-trapping using *N*-methyl-D-glucamine dithiocarbamate complex ($(MGD)_2$–Fe^{2+}), which reacts with NO to give a stable paramagnetic complex, $(MGD)_2$–Fe^{2+}–NO.[10] Since the $(MGD)_2$–Fe^{2+}–NO complex gives a typical broadened three-line signal in the ESR spectrum, the generation of NO in the sample solution could be detected from the change in the spectrum. A freshly prepared $(MGD)_2$–Fe^{2+} complex solution and ND micelles were mixed in a quartz cell (molar ratio: $[(MGD)_2$–$Fe^{2+}]/[\text{NTP unit}] = 20$), after which the mixed solution was irradiated for 1 h. Before irradiation, the mixed solution showed no significant peak in the ESR spectrum (Figure 2a). In contrast, the appearance of characteristic peaks corresponding to the formation of the paramagnetic $(MGD)_2$–Fe^{2+}–NO complex was observed after irradiation (Figure 2b). As a control, a solution of the $(MGD)_2$–Fe^{2+}

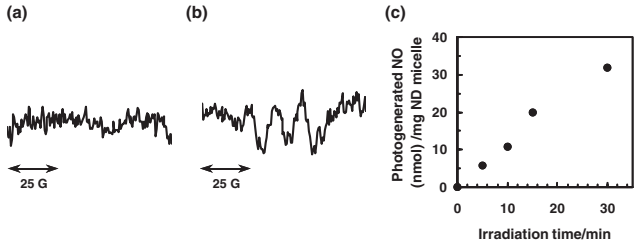

Figure 2. ESR spectra of the $(MGD)_2$–Fe^{2+}/ND micelle mixture (a) before and (b) after light irradiation. The spectra were recorded at modulation amplitude of 8.0 G. (c) Phototriggered NO generation from the ND micelles estimated by the Griess method. In all experiments, light irradiation was performed using a high-pressure mercury lamp and a glass filter (>315 nm, 54 mW cm^{-2}).

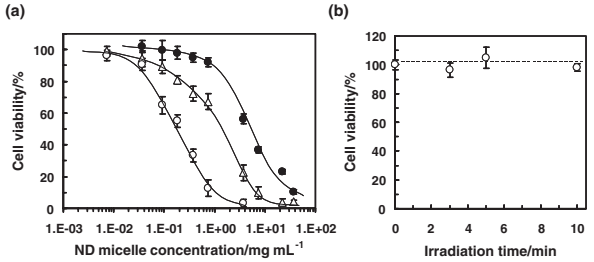

Figure 3. (a) Viability of HeLa cells treated with ND micelles at various concentrations with (open symbol) or without (filled circle) UV light irradiation. Light irradiation was performed using a mercury lamp and a band pass filter (330–385 nm, 34 mW cm^{-2}) for 3 min (open triangles) and 10 min (open circles). The plotted data are averages of five experiments ± SD. (b) Viability of HeLa cells without ND micelle treatment after UV light irradiation. Light irradiation was performed as above. The plotted data are averages of five experiments ± SD.

complex alone was irradiated with the same dose of light, and no significant change in ESR signal was observed (Figure S4).[9] This indicates the phototriggered NO generation from the ND micelles. Though NO is rather hydrophobic character, it might be released from the ND micelles through the PEG outer layer due to the high mobility of gaseous NO. Next, the amount of photogenerated NO was estimated by the Griess method, which can quantify the amount of both nitrite and nitrate ions resulting from NO oxidation and subsequent hydrolysis in aqueous solutions.[11] As shown in Figure 2c, the amount of photogenerated NO progressively increased with the increase in irradiation time, and after 30 min of irradiation, 31.9 nmol of NO was generated from 1 mg of ND micelles, corresponding to a reactivity of ca. 2% of the NTP units within the ND micelle core.

In order to assess the photoinduced NO-mediated antitumor effect of the ND micelles, in vitro examination was performed using HeLa cells.[9] Figure 3a shows the change in the viability of HeLa cells as a function of the concentration of ND micelles. Without UV light irradiation, the ND micelles showed a relatively lower cytotoxicity, and the 50% inhibitory concentration (IC_{50}) was found to be 4.9 mg mL^{-1}. This might be due to the existence of the biocompatible PEG outer layer at the surface of the ND micelles. Upon UV light irradiation, the cytotoxicity of the ND micelles was enhanced with the increase in irradiation time, and a significant decrease in the IC_{50} value of the ND micelles was confirmed (after 3-min irradiation (6.12 J cm^{-2}) $IC_{50} = 1.9$ mg mL^{-1}, after 10-min irradiation (20.4 J cm^{-2}) $IC_{50} = 0.2$ mg mL^{-1}). It should be noted that the fluence of the UV light employed in this experiment did not affect the viability of HeLa cells in the absence of ND micelles (Figure 3b), indicating that the photoinduced enhancement of the cytotoxicity of the ND micelles was caused by the photoproducts, NO and polymer-bonded oxyl radicals. Oxyl radical and its degradation products are known to exhibit no significant cytotoxicity,[5c] which strongly emphasize that the liberated NO works mainly to an antitumor effect in a photocontrolled manner.

In conclusion, the present study demonstrated the photocontrolled delivery of exogenous NO into target cells using a PEGylated polymer micelle containing NO photogenerative units within the core moiety. The amount of generated exogenous NO was confirmed to be sufficiently high to induce an antitumor effect, indicating the usability of this molecular system for NO-based tumor therapy. Finally, we wish to emphasize that the main objective of this study was to verify the effectiveness of a PEGylated polymer micelle-based NO photodonor system for tumor treatment. The wavelength of the light used in this study was within the UV region, which has an instinct limitation for in vivo applications. Further studies which address this issue, among others, are underway and the results will be reported elsewhere.

This work was supported by a Grant-in-Aid for Scientific Research (A) (No. 21240050) and World Premier International Research Center (WPI) Initiative on Materials Nanoarchitectonics (MANA) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

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