

Supporting Information

Photo-triggered cargo release from liposome chlorin e6-bearing pullulan hybrid nanoparticle *via* membrane permeabilization

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Materials and Methods

Materials

Calcein and chlorin e6 were purchased from Santa Cruz Biotechnology (Texas, USA). 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) and silica microparticles were obtained from Sigma Aldrich (Missouri, USA) and COREFRONT (Tokyo, Japan), respectively. 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and NBD-1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (NBD-PE) were purchased from NOF (Tokyo, Japan). Human mesenchymal stem cells (TAKARA BIO INC., Shiga, Japan) were maintained in Mesenchymal Stem Cell Growth Medium (TAKARA BIO INC.). Cell Counting Kit-8 and phosphotungstic acid were purchased from Dojindo (Kumamoto, Japan) and Nacalai Tesque (Kyoto, Japan).

Preparation of liposomes *via* extrusion methods

DOPC liposomes and cationic liposomes were prepared as previously reported. DOPC (50 mM) or a mixture of DOPC and cationic lipid (DOPC, 45 mM; cationic lipid, 5 mM) was dissolved in dry chloroform. The solvents were vaporized using a gentle stream of nitrogen. The resulting thin films were hydrated above the phase transition. Afterward, the suspensions were treated with an extruder using a porous membrane (50 nm mesh).

Preparation and characterization of liposome-Che6P nanogel hybrids

Liposomes (0.005, 0.05, 0.5, and 50 mM) and Che6P nanogels (0.5 mg•mL⁻¹) were mixed and incubated at 25°C. To confirm complexation, dynamic light scattering (DLS: Zetasizer Nano ZS; Malvern, UK) measurement and ζ-potential measurement were performed at each time point (0, 1, 2, 3, 6, and 24 h). Representative morphologies of the hybrids were observed using a transmission electron microscope (JEM-1400: JEOL Ltd.; Tokyo, Japan). The samples (liposome, 50 mM; Che6P nanogel, 0.5 mg•mL⁻¹) were loaded onto the surface of an ultrathin carbon deposited Cu grid, and the solvents were dried overnight. The hybrids were then stained with 1% phosphotungstic acid for 10 s. Fluorescence correlation spectroscopy was carried out using LSM800 (Carl Zeiss, Germany).

Singlet oxygen generation capacity of liposome-Che6P nanogel hybrids

Singlet oxygen generated by hybrid nanoparticles was detected using ABDA. DOPC liposome-Che6P nanogel hybrids (DOPC, 0.5 mM; Che6P, 0.1 mg•mL⁻¹) or cationic liposome-Che6P nanogel hybrids (DOPC, 0.45 mM; cationic lipid, 0.05 mM; Che6P, 0.1 mg•mL⁻¹) were mixed with ABDA solution dissolved in DMSO (25 μM). After oxygen was bubbled through the sample suspensions, photoirradiation was carried out for 60 mins using a xenon lamp (SX-UID500X, 500 W: Ushio Inc., Tokyo, Japan). The light power was fixed at 16 mW•cm⁻² (over 620 nm), and the absorption spectrum was measured at each time point (0, 7.5, 15, 30, and 60 min).

Preparation of calcein-loaded liposomes

Calcein-loaded liposomes were prepared as previously reported. After preparation of a thin layer using DOPC (50 mM) or a mixture of DOPC and cationic lipid (DOPC, 47.5 mM; cationic lipid, 2.5 mM), calcein solution (10 mM) was applied and vigorously mixed with a vortex mixer. The resulting suspensions were treated with freeze-thaw cycles at least eight times to produce unilamellar vesicles. To control their size distribution, the samples were treated with an extruder (50 nm). To remove free calcein, gel column chromatography was carried out using Sepharose400.

Photo-triggered payload release from liposome-Che6P nanogel hybrids

Liposome-Che6P nanogel hybrids were prepared using calcein-loaded liposomes. The hybrids were irradiated for seven hours using a xenon lamp. The light power was fixed at 16 mW•cm⁻² (over 620 nm). The fluorescence spectra and DLS of the resulting dispersion were measured at each time point (ex, 490 nm).

Photo-triggered payload release from gelatin hydrogel comprising liposome-Che6P nanogel hybrids

A dispersion of the DOPC liposome-Che6P nanogel hybrids (Che6P, 0.1 mg•mL⁻¹; DOPC, 0.1 mM) was mixed with a gelatin dispersion (10 wt%). Subsequently, 25% glutaraldehyde was added to the resulting dispersion and incubated overnight at 4°C. Unreacted glutaraldehyde was removed via immersion in saline for 24 h. After the supernatant was

exchanged with fresh saline, the hydrogels were exposed to light using a xenon lamp. The light power was fixed at $16 \text{ mW}\cdot\text{cm}^{-2}$ (over 620 nm). Fluorescence spectra of supernatant were measured at each time point (ex, 490 nm).

Cytotoxicity of gelatin hydrogels comprised of liposome-Che6P nanogel hybrids

Mesenchymal stem cells were seeded on 12-well plates and incubated overnight (approximately 18 h). After the medium change, gelatin hydrogel containing liposome-Che6P nanogel hybrids was placed on the 12-well plate. After 24 h of incubation, the systems were irradiated with a xenon lamp (MAX-301, 300 W; Asahi Spectra Co., Ltd, Tokyo, Japan) for 6 h. The light power was fixed at $16 \text{ mW}\cdot\text{cm}^{-2}$ (over 620 nm). After an additional 18 h, a solution of Cell Counting Kit-8 was added to each well. The absorbance at 450 nm was measured using a microplate reader.

Photo-triggered payload release from gelatin hydrogels containing liposome-Che6P nanogel hybrids *in vitro*

Human mesenchymal stem cells were seeded on glass-bottom dishes and incubated overnight. The cells were coincubated with gelatin hydrogel containing DOPC-Che6P nanogel hybrids, and photoirradiation was conducted for an initial 6 h. The light power was fixed at $16 \text{ mW}\cdot\text{cm}^{-2}$ (over 620 nm). After an additional 18 h of incubation, the cells were observed using confocal laser scanning microscopy (LSM700, Carl Zeiss, Germany).

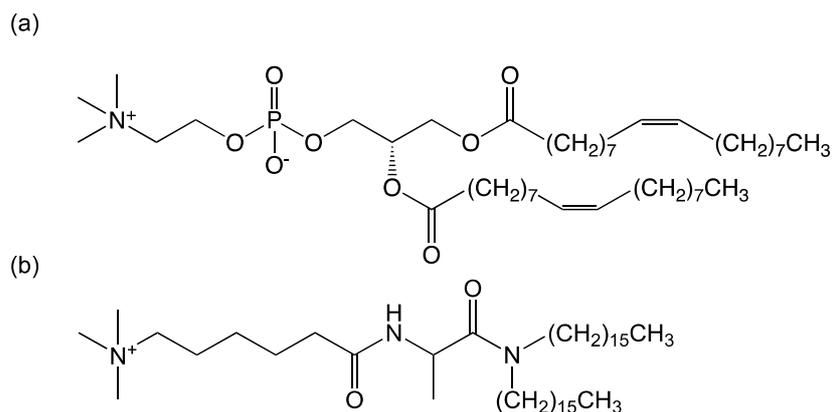


Fig. S1. Chemical formula of DOPC (a) and cationic lipid (b).

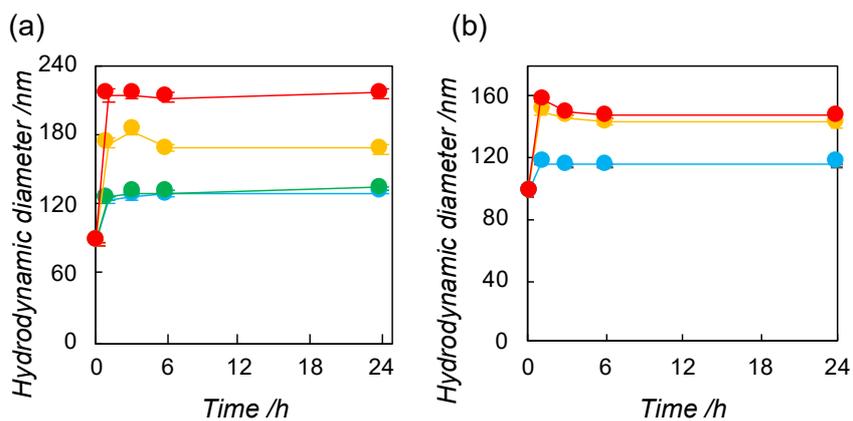


Fig. S2. Changes in distribution of the hydrodynamic diameter of hybrids using (a) DOPC liposome and (b) cationic liposome (DOPC, 95%; cationic lipid, 5%). Concentration of Che6P was fixed at $0.5 \text{ mg}\cdot\text{mL}^{-1}$ and concentration of DOPC was varying (0.005, red; 0.05, yellow; 0.5, green; and 1 mM, blue). Concentration of Che6P was fixed at $0.5 \text{ mg}\cdot\text{mL}^{-1}$ and concentration of lipid was varying (0.005, red; 0.05, yellow; 0.5, and 1 mM, blue). Data represent mean \pm SD.

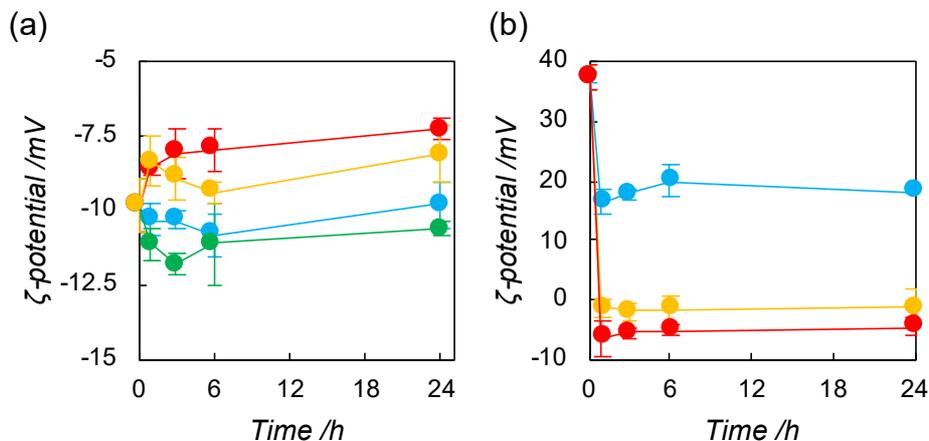


Fig. S3. Changes in ζ -potential of hybrids using (a) DOPC liposome and (b) cationic liposome (DOPC, 95%; cationic lipid, 5%). Concentration of Che6P was fixed at $0.5 \text{ mg}\cdot\text{mL}^{-1}$ and concentration of DOPC was varying (0.005 , red; 0.05 , yellow; 0.5 , green; and 1 mM , blue). Concentration of Che6P was fixed at $0.5 \text{ mg}\cdot\text{mL}^{-1}$ and concentration of lipid was varying (0.005 , red; 0.05 , yellow; 0.5 , and 1 mM , blue). Data represent mean \pm SD.

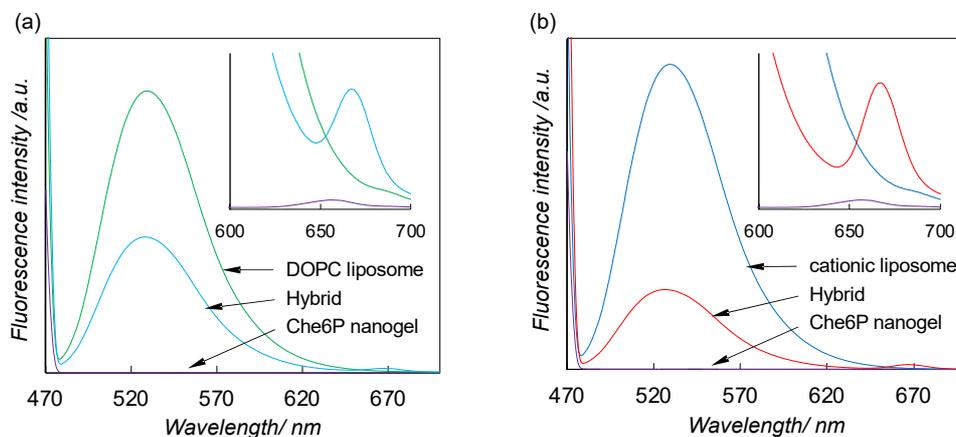


Fig. S4. Fluorescence resonance energy transfer between chlorin e6 and fluorescently labelled lipid (NBD-PE). Hybrid particles were prepared by mixing Che6P nanogel ($0.1 \text{ mg}\cdot\text{mL}^{-1}$) and liposome containing NBD-PE (a: DOPC liposome; NBD-PE, $4 \mu\text{M}$; DOPC, 1 mM ; b: cationic liposome, NBD-PE, $4 \mu\text{M}$; DOPC 0.95 mM ; cationic lipid, 0.05 mM). The samples were excited at 460 nm . Green, purple, and blue curve in (a) represent NBD-PE containing liposome, Che6P nanogel, and hybrid particle, respectively. Deep blue, purple, and red curve in (b) represent NBD-PE containing cationic liposome, Che6P nanogel, and hybrid particle, respectively.

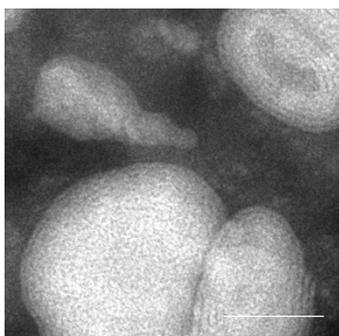


Fig. S5. Representative morphological images of DOPC liposome-Che6P nanogel hybrids. Scale bar represent 100 nm.

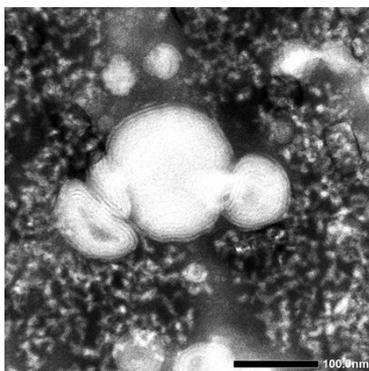


Fig. S6. Representative morphological images of cationic liposome-Che6P nanogel hybrids. Scale bar represents 100 nm.

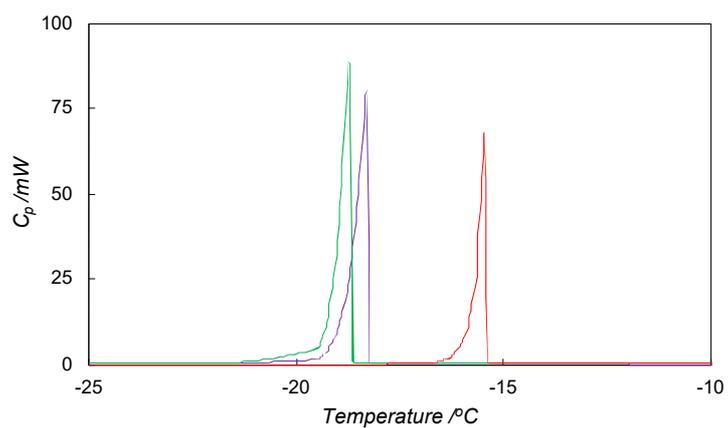


Fig. S7. DSC thermograms showing the effect of Che6P nanogels on the phase transition for cationic liposome. (cationic liposome, green; cationic liposome/Che6P nanogel hybrid, purple; cationic liposome containing chlorin e6, red).

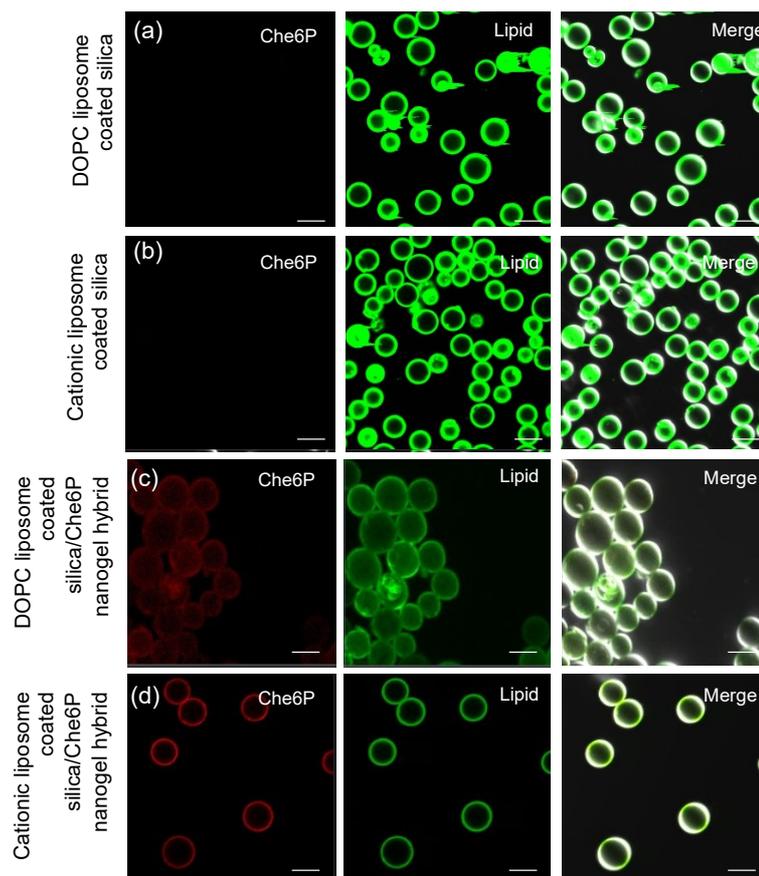
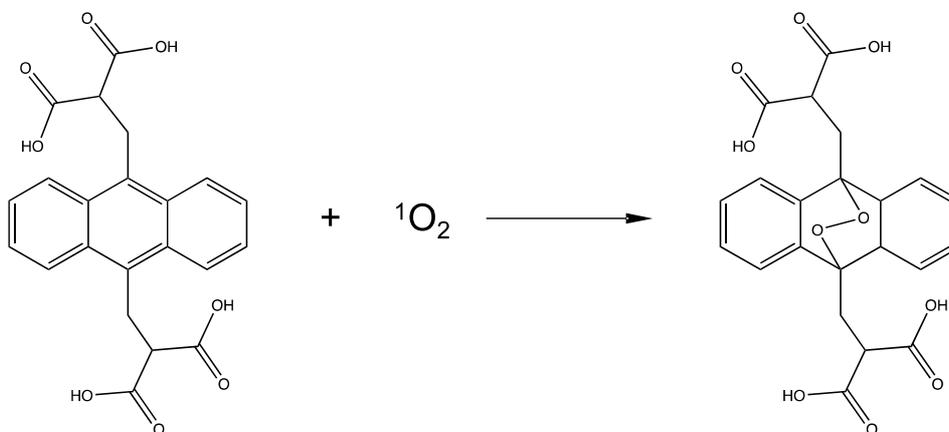


Fig. S8. (a) Fluorescence images of lipid membrane coated silica microparticle hybrid (red, Che6P; green, lipid; yellow, merge). (b) Fluorescence images of cationic lipid coated silica microparticle hybrid (red, Che6P; green, lipid; yellow, merge). (c) Fluorescence images of Che6P nanogel-lipid membrane coated silica microparticle hybrid (red, Che6P; green, lipid; yellow, merge). (d) Fluorescence images of Che6P nanogel-cationic lipid coated silica microparticle hybrid (red, Che6P; green, lipid; yellow, merge). Scale bar represents 10 μm .



Scheme S1. Conversion of ABDA to endoperoxide via oxidation by $^1\text{O}_2$.

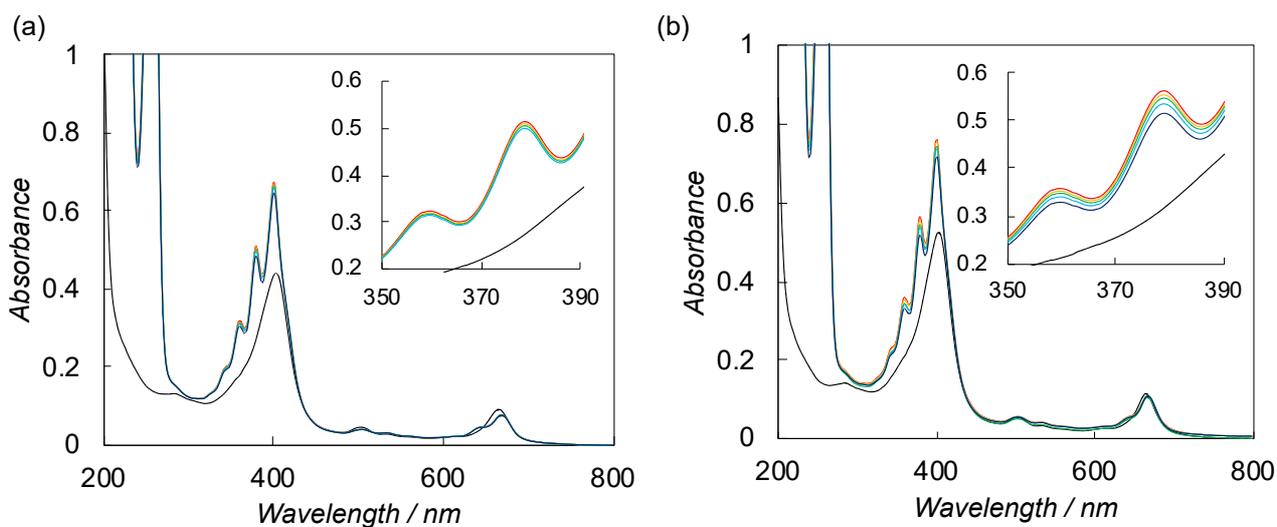


Fig. S9. Representative UV absorption spectrum in bleaching of ABDA via oxidation with $^1\text{O}_2$ generated using (a) DOPC liposome-Che6P nanogel hybrid and (b) cationic liposome-Che6P nanogel hybrids. The polymer concentration was fixed at $0.1 \text{ mg}\cdot\text{mL}^{-1}$.

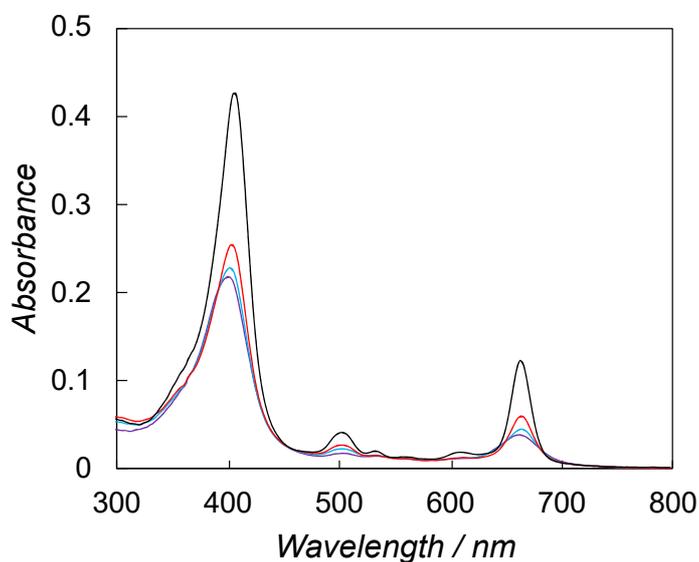


Fig. S10. UV-Vis spectrum of Che6P nanogel hybrid. (Che6P nanogel, purple; Che6P nanogel-DOPC liposome hybrid, blue; Che6P nanogel-cationic liposome hybrid, red; chlorin e6 dissolved in DMSO, black).

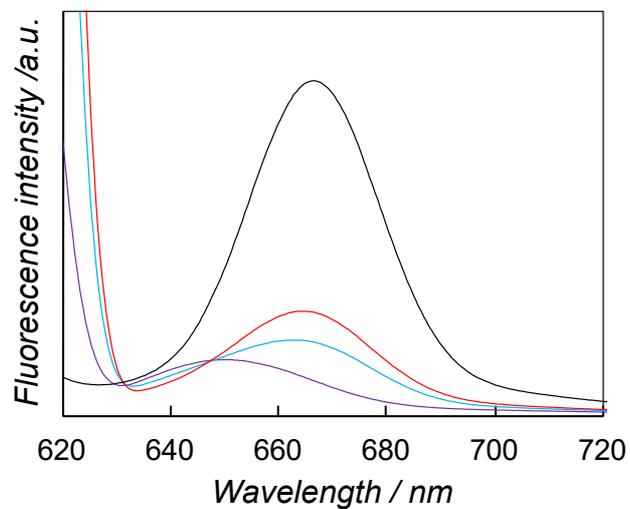


Fig. S11. Fluorescence spectrum of Che6P nanogel hybrid. (Che6P nanogel, purple; Che6P nanogel-DOPC liposome hybrid, blue; Che6P nanogel-cationic liposome hybrid, red; chlorin e6 dissolved in DMSO, black).

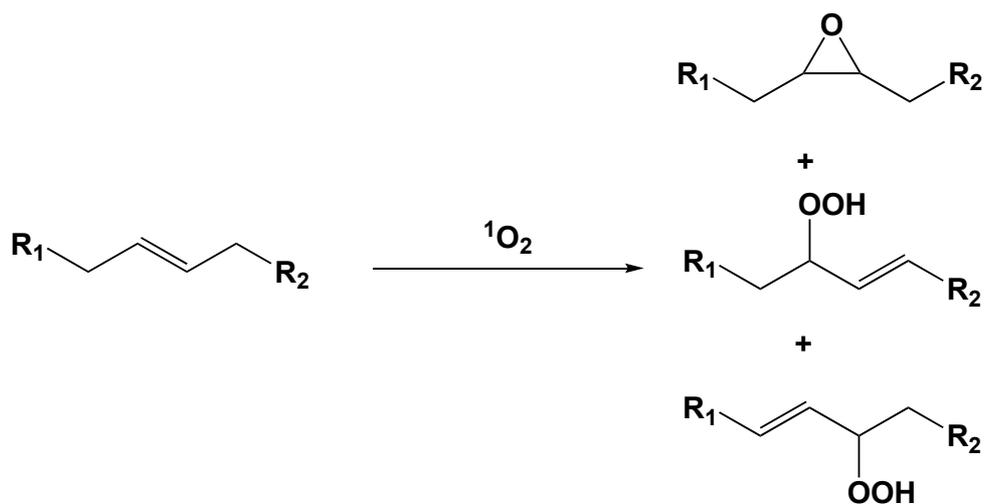


Fig. S12. Estimated reaction and chemical formulas of oxidized DOPC.

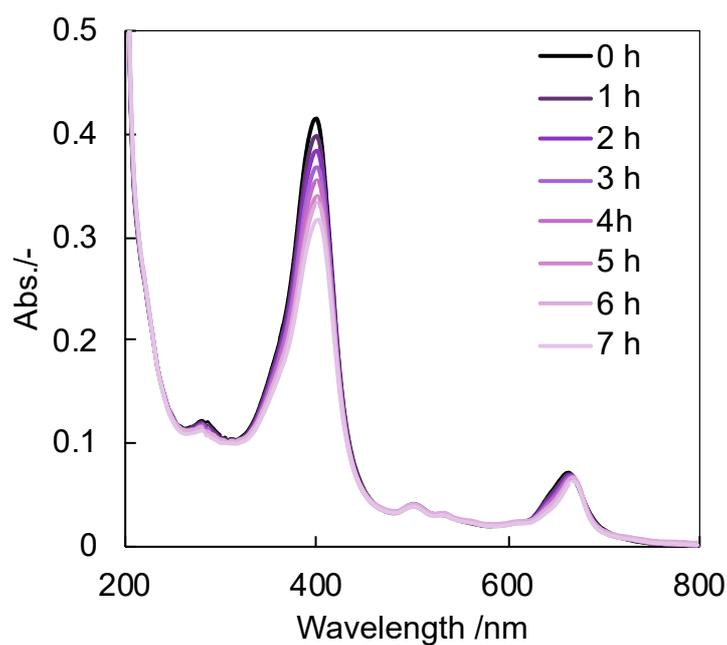


Fig. S13. Stability of chlorin e6 during photo-irradiation.

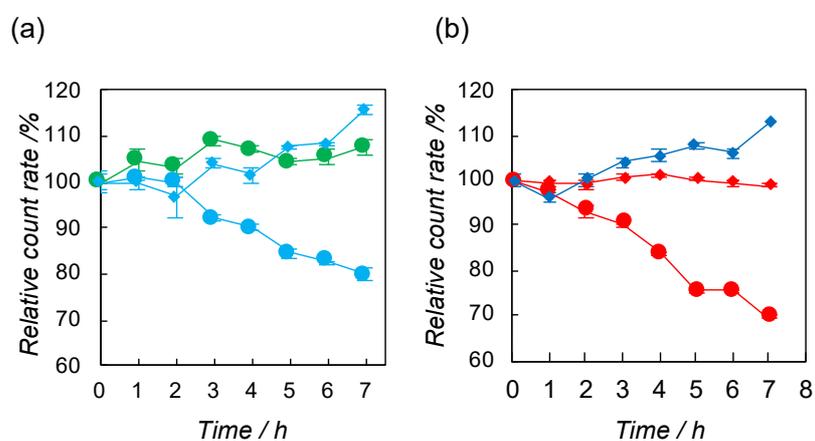


Fig. S14. Count rate change of nanoparticles *via* photoirradiation using (a) DOPC liposome and (b) cationic liposome. Count rate change of nanoparticles *via* photoirradiation (DOPC liposome, green; Che6P nanogel-DOPC liposome hybrid, blue). Circle symbol and diamond symbol indicate with irradiation group and without irradiation group, respectively. (cationic liposome, deep blue; Che6P nanogel-cationic liposome hybrid, red). Circle symbol and diamond symbol indicate with irradiation group and without irradiation group, respectively.

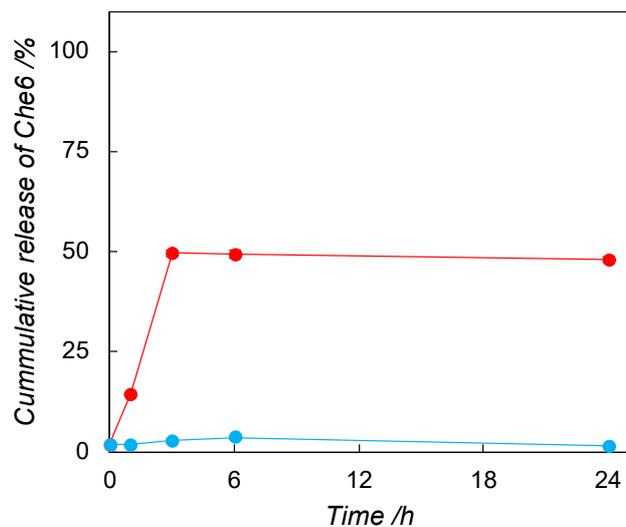


Fig. S15. Release of chlorin e6 molecules from hybrid hydrogel containing Che6P/DOPC (red) and Che6P nanogel/DOPC (blue). Data represent mean \pm standard deviation (n=3).

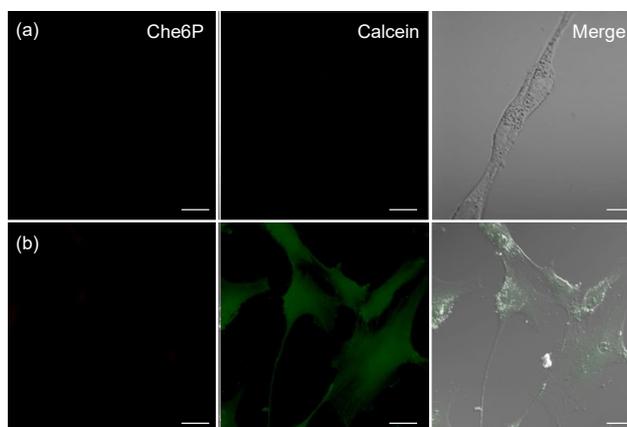


Fig. S16. Cellular uptake of Che6P nanogel (red) and calcein (green) with irradiation. The cells were co-incubated with hydrogel containing DOPC liposome/Che6P nanogel hybrid without irradiating light for 30 min (a) and with irradiating light (b). After additional 23.5 h, cellular uptake was confirmed by confocal laser scanning microscopy. Scale bar represents 10 μ m.

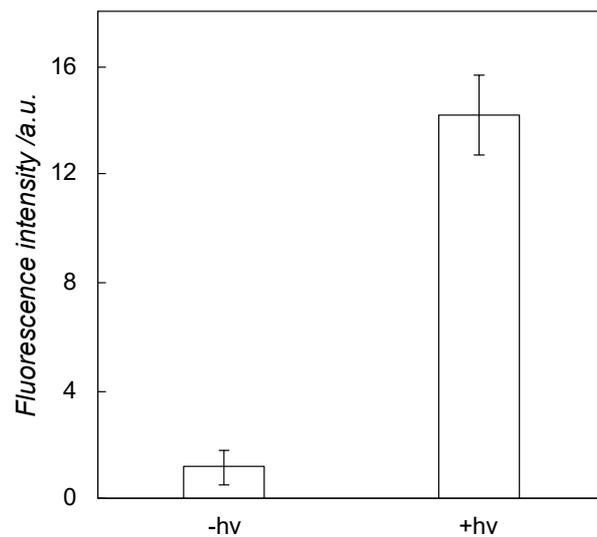


Fig. S17. Cellular uptake amount of released Calcein from gelatin hydrogel containing DOPC-Che6P nanogel hybrid. hMSC were co-incubated with the hydrogel for 24 h and photoirradiation was carried out initial 6 h. After additional 18 h, the cells were analyzed by flow cytometry (N=3).