Supplementary Information

Calcium Phosphate-Based Organic-Inorganic Hybrid Nanocarriers with pH-Responsive On/Off Switch of Photodynamic Therapy

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Supplementary Methods

X-ray diffraction (XRD)

To evaluate crystal phase of calcium phosphate (CaP) in the nanocarrier, CaP incorporating chlorin e6 (Ce6) was prepared in the absence of poly(ethylene glycol)-*b*-poly(L-aspartic acid) (PEG-PAsp). Solution of 2.5 M $CaCl_2$ was diluted in 10 mM Tris/HCl buffer (pH 7.6) (2 mL: 18 mL). This solution was added into the same volume of another solution containing Ce6 (100 μg/mL) in 50 mM HEPES buffer containing 6 mM Na_2HPO_4 and 140 mM NaCl (pH 7.1) to prepare CaP incorporating Ce6. The mixed aqueous solution was then incubated at 37 °C for at least 30 min. For hydrothermal synthesis, the CaP incorporating Ce6 was autoclaved at 120 °C for 60 min, using LSX-700 (TOMY Seiko Co., Ltd., Tokyo, Japan). The CaP incorporating Ce6 with or without hydrothermal synthesis was spun down, and the supernatant was carefully removed. These precipitates were washed with ethanol, and centrifuged, followed by removal of the supernatant. The prepared CaP incorporating Ce6 was then put in an aluminum mold. After drying the samples by air, the X-ray diffraction was measured with Cu Kα radiation using XRD-DSC II (RIGAKU Corporation, Tokyo, Japan).

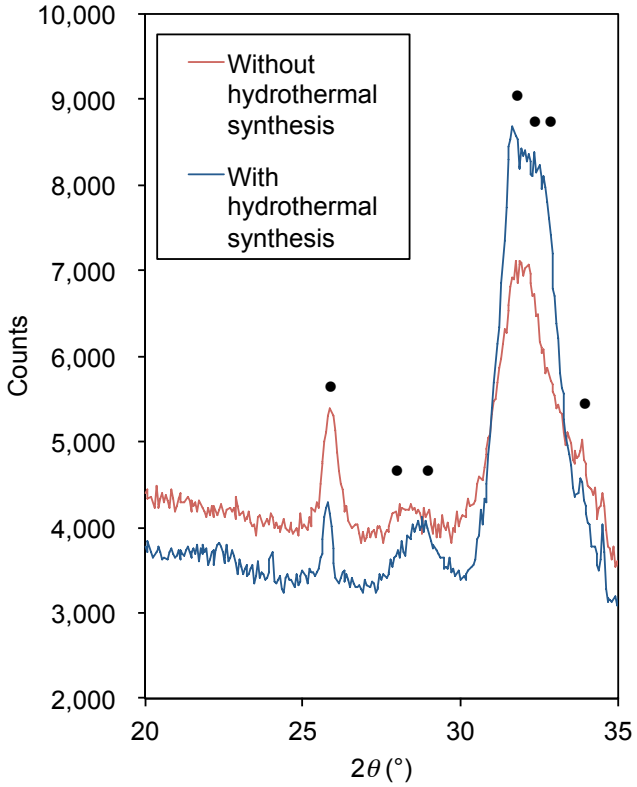
Fourier transform infrared (FT-IR) spectroscopy

CaP incorporating Ce6 was prepared and purified in the same method for preparation of the CaP for XRD measurement. The CaP incorporating Ce6 was dried by air, and FT-IR spectrum was measured using FT/IR-4600 (JASCO Corporation, Tokyo, Japan).

Validation of the integrity of Ce6 using ^{1}H nuclear magnetic resonance (NMR) spectroscopy

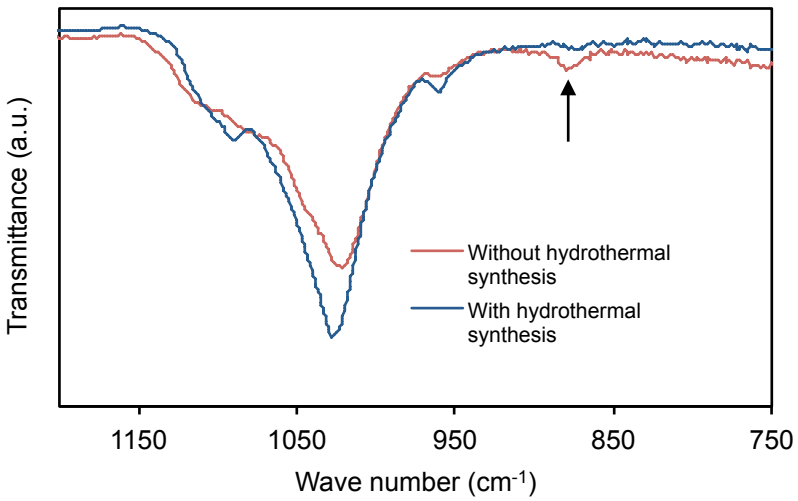
CaP incorporating Ce6 was prepared and purified in the same method for preparation of the CaP for XRD measurement. After vacuum-drying overnight, the hydrothermally synthesized CaP incorporating Ce6 was dissolved in the mixture of 0.5 N deuterium chloride and dimethyl sulfoxide-d_6 (v/v=1), and characterized by ^{1}H NMR spectroscopy using AVANCE III 400 (Bruker BioSpin K.K., Kanagawa, Japan).

Supplementary Figures S1–S3



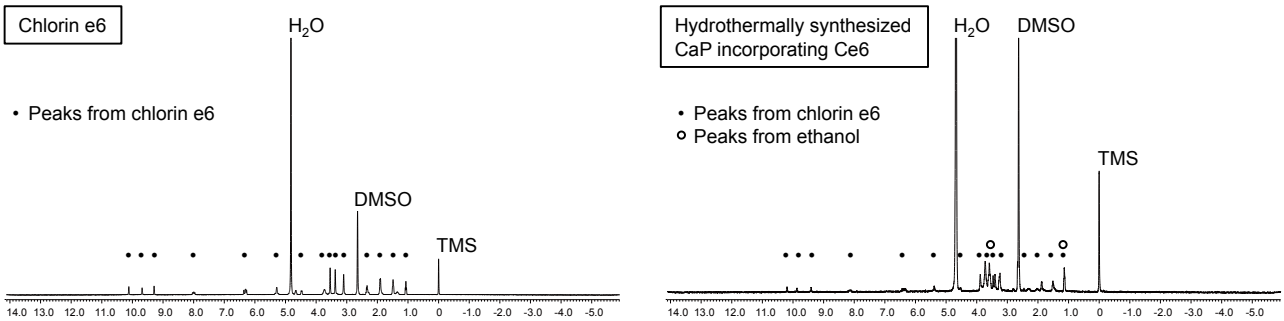
Supplementary Figure S1. XRD patterns of CaP incorporating Ce6.

XRD patterns of CaP incorporating Ce6 with/without hydrothermal synthesis were measured on aluminum molds. Closed circles highlight typical peaks from hydroxyapatite.



Supplementary Figure S2. FT-IR spectra of CaP incorporating Ce6.

FT-IR spectra of CaP incorporating Ce6 with/without hydrothermal synthesis were measured in solid form. The arrow indicates the peak corresponding to CO_3^{2-}.



Supplementary Figure S3. ^{1}H NMR of Ce6 encapsulated in hydrothermally synthesized CaP.

^{1}H NMR spectra of Ce6 and Ce6 encapsulated in hydrothermally synthesized CaP were measured in the mixture of 0.5 N deuterium chloride and dimethyl sulfoxide-d_6 (v/v=1). Closed circles and open circles highlight the chemical shifts of chlorin e6 and ethanol that was used to wash the CaP, respectively.

Captions for Supplementary Movies

Supplementary Movie S1. Intravital microscopic imaging of the bloodstream in the mouse injected with CaPCe6.

CaPCe6 was intravenously injected into a mouse and its blood vessels in the ear lobe were monitored using IVRTCLSM at 30 fps. Fluorescence of Ce6 and Dio-labeled red blood cells is shown in red and green, respectively.

Supplementary Movie S2. Intravital microscopic imaging of the bloodstream in the mouse injected with Ce6.

Ce6 was intravenously injected into a mouse and its blood vessels in the ear lobe were monitored using IVRTCLSM at 30 fps. Fluorescence of Ce6 and Dio-labeled red blood cells is shown in red and green, respectively.