Supporting Information

Visible-Light-Responsive Photocatalyst of Graphitic Carbon Nitride for Pathogenic Biofilm Control

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Disinfection of planktonic *Escherichia coli* (*E. coli*) with g-C_3N_4 under the irradiation of white light-emitting diodes (LEDs)

E. coli (ATCC 11775) was firstly cultured in Luria-Bertani broth (LB, 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 1 L of ultrapure water) at 37 °C with mixing (120 rpm on a rotary shaker) overnight. The bacterial cells in their late-exponential phase were harvested by centrifugation at 3000 rpm and resuspended in phosphate-buffered saline (PBS, pH 7.4) to prepare a bacterial suspension (OD_{600} = 0.5). Next, 25 mL of the bacterial suspension was mixed with 0.0015 g of $MCB_{0.07}$ powder in a sterile glass beaker (0.06 g L^{-1} of photocatalyst loading), and the beaker was placed under a white LED lamp (7 W) for bacterial inactivation by photocatalysis. The distance between the surface of the bacterial suspension and the LED lamp was maintained at 15 cm. The spectral irradiance of the light source was recorded in **Figure S1**, and the photon flux and the optical power density were 161.66 μmol s^{-1} m^{-2} and 4359.48 μW cm^{-2}, respectively, considering the photons that could be utilized in photocatalysis ($MCB_{0.07}$ uses photons with a wavelength shorter than 460 nm). Bacterial suspension samples were withdrawn from the beaker every half an hour. The samples were duplicated, diluted in series with the PBS, and plate counting was conducted to determine bacterial viability. The disinfection performance of $MCB_{0.07}$ powder under LED light irradiation was shown in **Figure S2**. Dark and light control experiments were also performed under the same experimental conditions, in the presence of the photocatalyst but no light and in the presence of light but no photocatalyst, respectively.

ROS Measurement

·OH Measurement. The reaction between *para*-chlorobenzoic acid (*p*-CBA) (Sigma-Aldrich, 98%) and ·OH could be described in Eq. S1; the steady-state ·OH concentration can be similarly calculated by Eq. S2:[1]

$$-\frac{d[p\text{-CBA}]}{dt} = k_{p\text{-CBA}}[\cdot\,\mathrm{OH}]_{SS}[p\text{-}\,\mathrm{CBA}] \quad \text{(S1)}$$

$$[\cdot\,\mathrm{OH}]_{SS} = \frac{k_{ex}}{k_{p-\mathrm{CBA}}} \quad \text{(S2)}$$

where $[\cdot\,\mathrm{OH}]_{SS}$ is the steady-state concentration of ·OH, k_{ex} is the experimentally observed pseudo-first-order rate constant, and $k_{p\text{-CBA}}$ is the second-order reaction rate for ·OH and *p*-CBA reaction, $k_{p\text{-CBA}} = 5.2 \times 10^9\ \mathrm{M^{-1}s^{-1}}$.[2] *p*-CBA concentrations were measured by high performance liquid chromatography (HPLC, Shimadzu LC-20AT Prominence) with a Shimadzu C18 column (4.6 × 50 mm, 1.8 μm particle size) at 230 nm using UV absorbance. A mobile phase (acetonitrile/water (14:86 by volume) containing 40 mM phosphate buffer (pH = 7)) with a flow rate of 1 mL min^{-1} was applied for the separation of *p*-CBA. The detection limit of $[\cdot\,\mathrm{OH}]_{SS}$ is on the order of 10^{-15} M, which was calculated from the reaction kinetics of *p*-CBA and ·OH (Eq. S1), by assuming 5% of *p*-CBA degradation is discernable in the HPLC analysis after a 1 h photocatalytic experiment ($k_{ex} = \frac{ln(C_0/C)}{t}$, where C_0/C=1/0.95, and t = 1 h).[3]

$^1O_2$ Measurement. The steady-state concentration of $^1O_2$ was calculated by measuring the consumption of furfuryl alcohol (FFA) (Sigma-Aldrich, 98%). The reaction between FFA and $^1O_2$ is described in Eq. S3.[4]

$$-\frac{d[FFA]}{dt} = k_{FFA}\left[{}^1O_2\right]_{SS}[FFA] \quad \text{(S3)}$$

$$-\frac{d[FFA]}{dt} = k_{ex}[FFA] \quad \text{(S4)}$$

$$\left[{}^1O_2\right]_{SS} = \frac{k_{ex}}{k_{FFA}} \quad \text{(S5)}$$

where $\left[{}^1O_2\right]_{SS}$ is the steady-state concentration of $^1O_2$, k_{ex} is the experimentally observed pseudo-first-order rate constant, and k_{FFA} is the second-order reaction rate for $^1O_2$ and FFA reaction, $k_{FFA} = 1.2 \times 10^8\ \mathrm{M^{-1}s^{-1}}$.[5] FFA concentration was measured by HPLC (Shimadzu LC-20AT Prominence) with a Shimadzu C18 column (4.6 × 50 mm, 1.8 μm particle size) at 218 nm. The isocratic mobile phase for FFA separation was methanol and water (15:85 by volume) acidified with orthophosphoric acid (0.1%), and the flow rate was 1 mL min^{-1}. The estimated detection limit for $\left[{}^1O_2\right]_{SS}$ is on the order of 10^{-13} M.[6]

$O_2^{-}\cdot$ Measurement. The steady state concentration of $O_2^{-}\cdot$ was calculated by monitoring the loss of XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide, Cayman chemical). XTT can react with $O_2^{-}\cdot$ to form water-soluble XTT-formazan ($XTT^{-}\cdot$) with the maximum absorption at 470 nm (UV-vis spectrophotometer, Hach DR6000).

$$O_2^- \cdot + XTT \rightarrow XTT^- \cdot \quad \text{(S6)}$$

$$[XTT] = [XTT]_{initial} - [XTT^- \cdot] \quad \text{(S7)}$$

$$-\frac{d[XTT]}{dt} = k_{XTT}[O_2^- \cdot]_{SS}[XTT] \quad \text{(S8)}$$

$$-\frac{d[XTT]}{dt} = k_{ex}[XTT] \quad \text{(S9)}$$

$$[O_2^- \cdot] = \frac{k_{ex}}{k_{XTT}} \quad \text{(S10)}$$

where $[XTT]_{initial} = 5 \times 10^{-4}$ M, $[O_2^- \cdot]_{SS}$ is the steady-state concentration of $O_2^- \cdot$, k_{ex} is the experimentally observed pseudo-first-order rate constant, and k_{XTT} is the second-order reaction rate for $O_2^- \cdot$ and XTT reaction, $k_{\mathrm{XTT}} = 8.6 \times 10^4\ \mathrm{M^{-1}s^{-1}}$.[7] The concentration of $XTT^- \cdot$ was calculated from light absorption (ABS), the molar adsorption coefficient of $XTT^- \cdot$ ($\varepsilon_{470} = 21.6\ \mathrm{mM^{-1}cm^{-1}}$), and the light penetration length in the spectrophotometric analysis (L=1 cm).[7]

$$[XTT^- \cdot] = \frac{ABS}{\varepsilon L} \quad \text{(S11)}$$

The detection limit can reach as low as 10^{-13} M in our study.[3]

H_2O_2 Measurement. The accumulated concentration of H_2O_2 was measured by the DPD method.[8] DPD reagent was prepared weekly by dissolving 0.1 g of *N,N*-diethyl-1,4-phenylenediammonium sulfate (DPD, 98% Sigma-Aldrich) in 10 mL of 0.1 N H_2SO_4 and stored in the dark at 4 °C. POD reagent was prepared weekly by dissolving 10 mg of peroxidase product from horseradish (Type II, Sigma) in 10 mL of ultrapure water and stored in the dark at 4 °C. For analysis, a 400 µL aliquot of the sample was mixed with 100 µL of 25 mM phosphate buffer (pH 6), 15 µL of the DPD reagent, and 15 µL of the POD reagent. Absorbance was measured at 551 nm (UV-vis spectrophotometer, Hach DR6000) after mixing for 1 min and resulting H_2O_2 concentration was determined via a calibration curve generated with known standards of H_2O_2. The detection limit was determined by the standard curve, which is on the order of 10^{-7} M.[3]

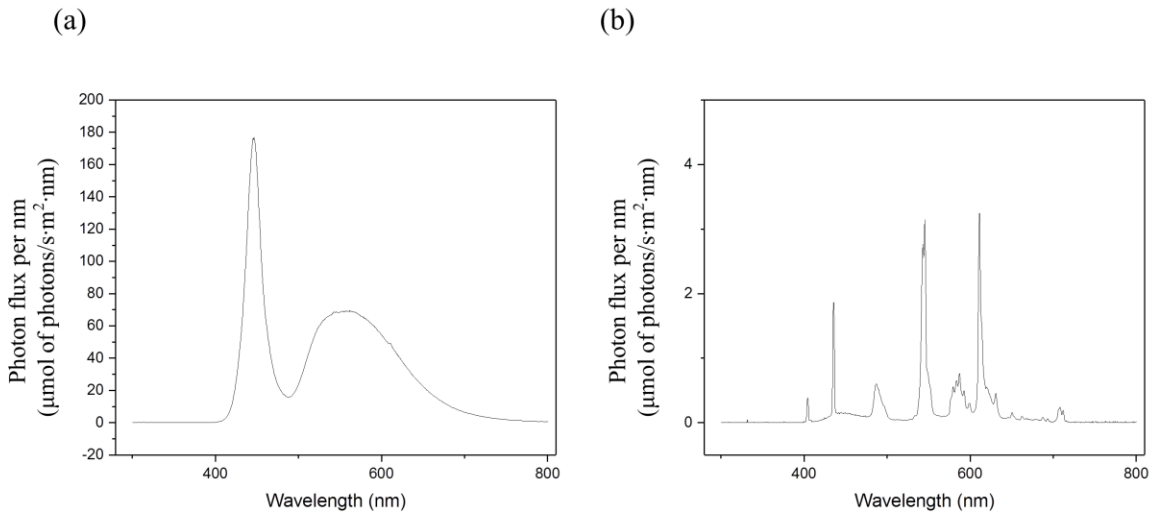

Figure S1. Spectrum of (a) white LED light and (b) weak visible indoor light.

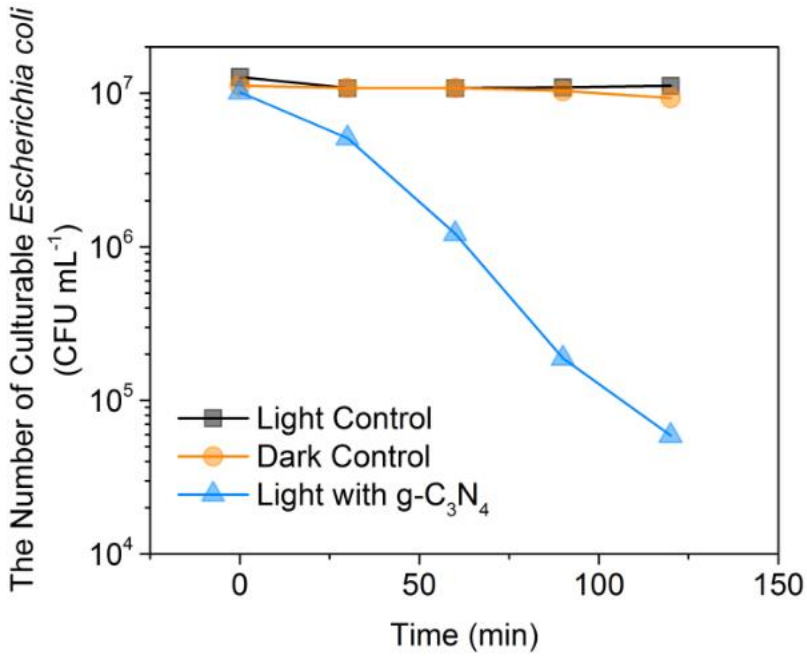

Figure S2. Disinfection of planktonic *E. coli* under white LED light irradiation in PBS. Dark and light control experiments represent the study in the presence of the photocatalyst but no light and in the presence of light but no photocatalyst, respectively. CFU represents colony forming units.

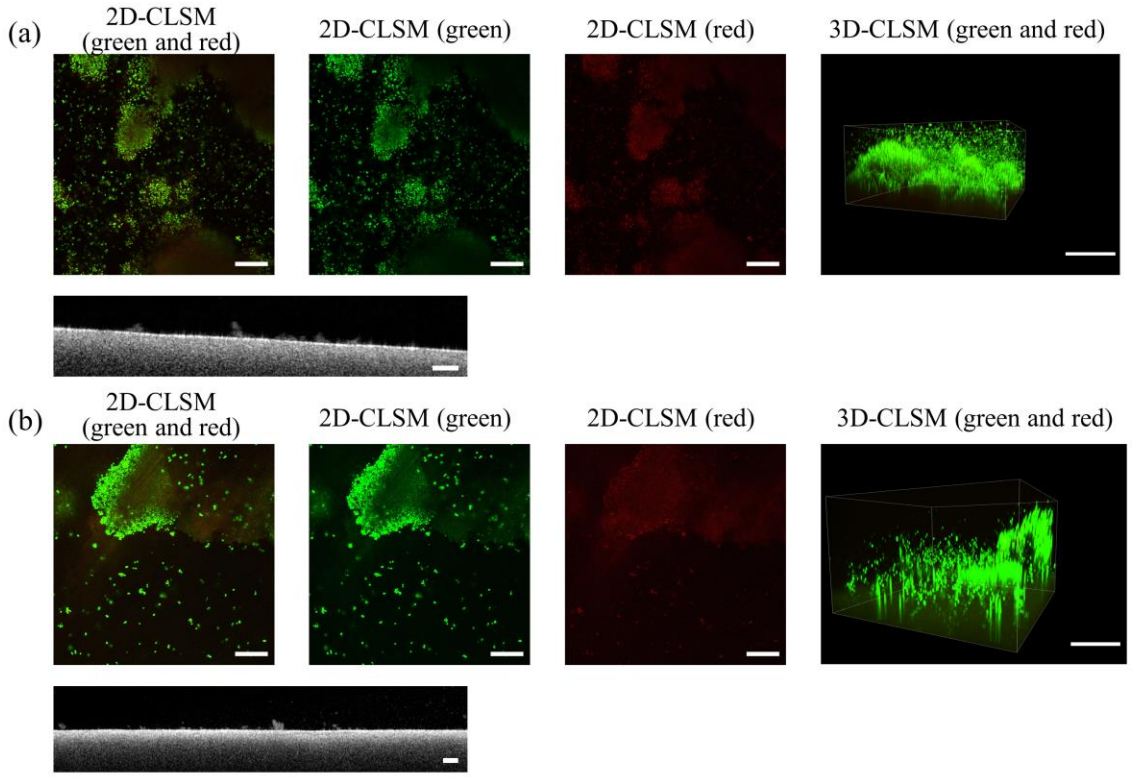

Figure S3. Mature *S. epidermidis* biofilm eradication from the g-C_3N_4 coupon surface under weak visible indoor light irradiation in 10-fold diluted TSB. (a) Mature biofilms grown in TSB in the dark for 3 days and (b) mature biofilms exposed to weak indoor light for 3 days in TSB. 10-fold diluted TSB was replenished daily. The scale bars in 2D confocal laser scanning microscopic (CLSM), 3D CLSM, and optical coherence tomographic (OCT) images are 20, 50, and 100 μm, respectively.

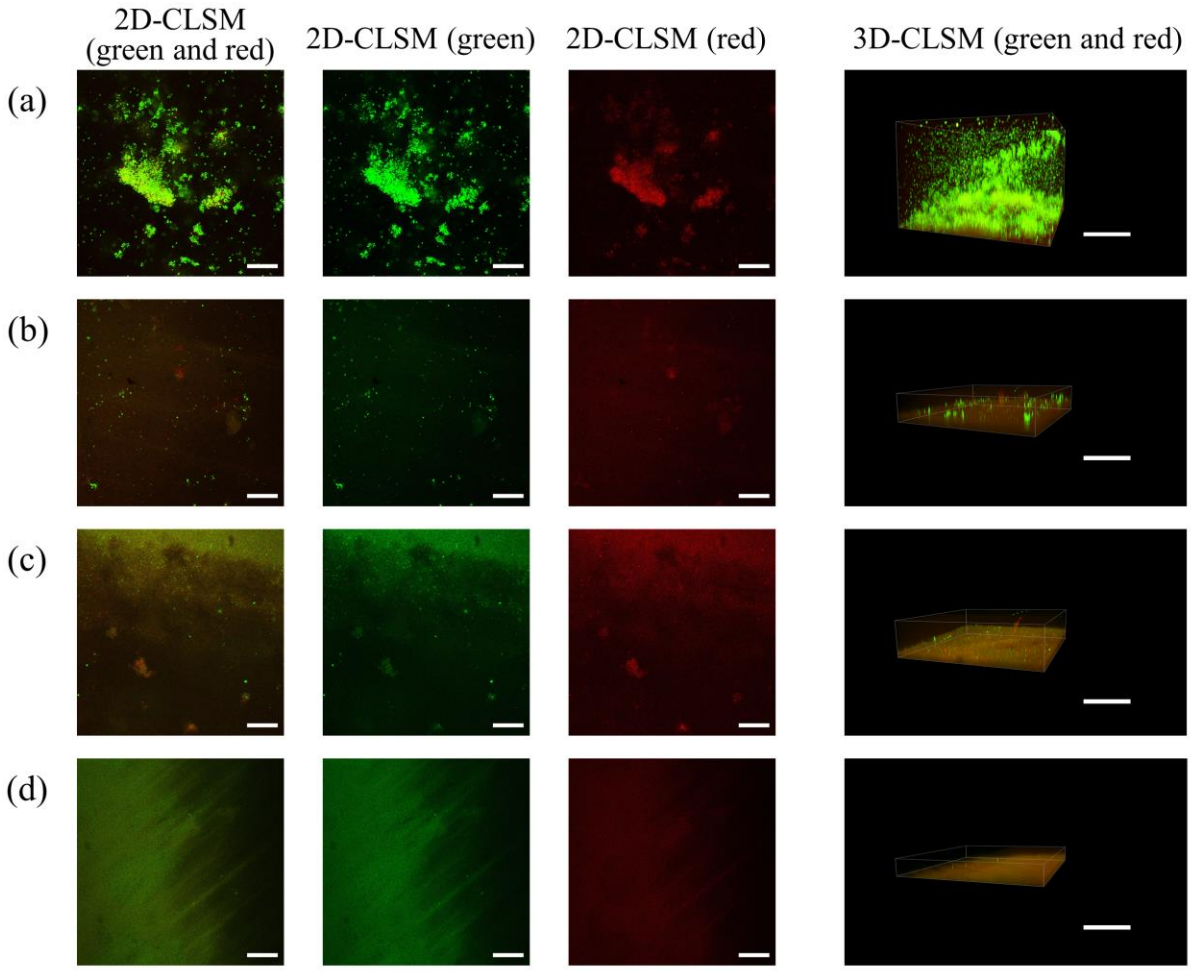

Figure S4. Mature *S. epidermidis* biofilm eradication from the g-C_3N_4 coupon surface under weak visible indoor light irradiation in PBS. (a) Mature biofilms grown in 10-fold diluted TSB in the dark for 3 days, (b) mature biofilms exposed to weak indoor light for 1 day in PBS, (c) mature biofilms exposed to weak indoor light for 2 days in PBS, and (d) mature biofilms exposed to weak indoor light for 3 days in PBS. The scale bars in 2D confocal laser scanning microscopic (CLSM) and 3D CLSM images are 20 and 50 μm, respectively.

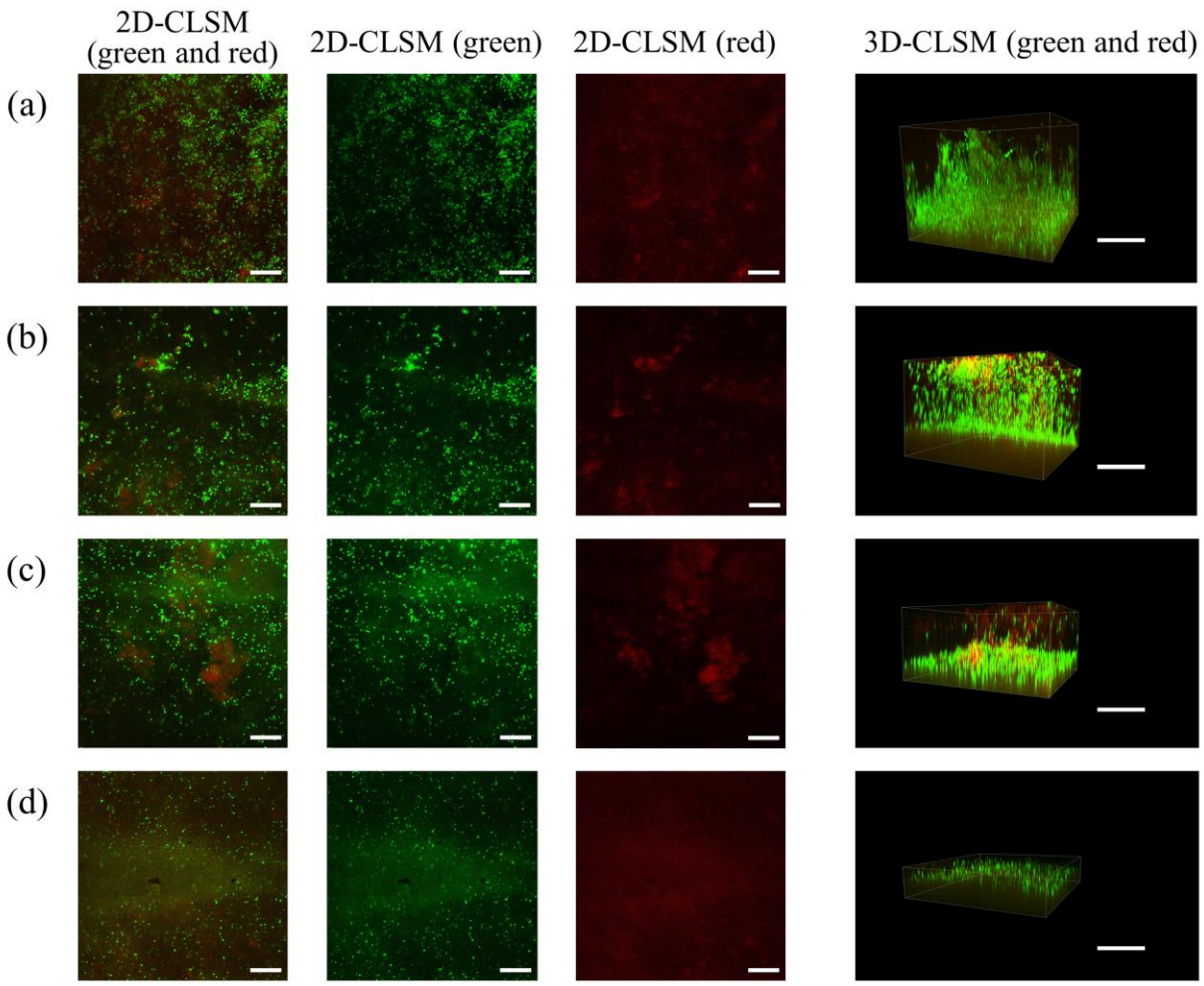

Figure S5. Viability of mature *S. epidermidis* biofilms on the g-C_3N_4 coupon surface in PBS in the dark. (a) Mature biofilms cultured in 10-fold diluted TSB in the dark for 3 days, (b) mature biofilms in PBS in the dark for 1 day, (c) mature biofilms in PBS in the dark for 2 days, and (d) mature biofilms in PBS in the dark for 3 days. The scale bars in 2D confocal laser scanning microscopic (CLSM) and 3D CLSM images are 20 and 50 μm, respectively

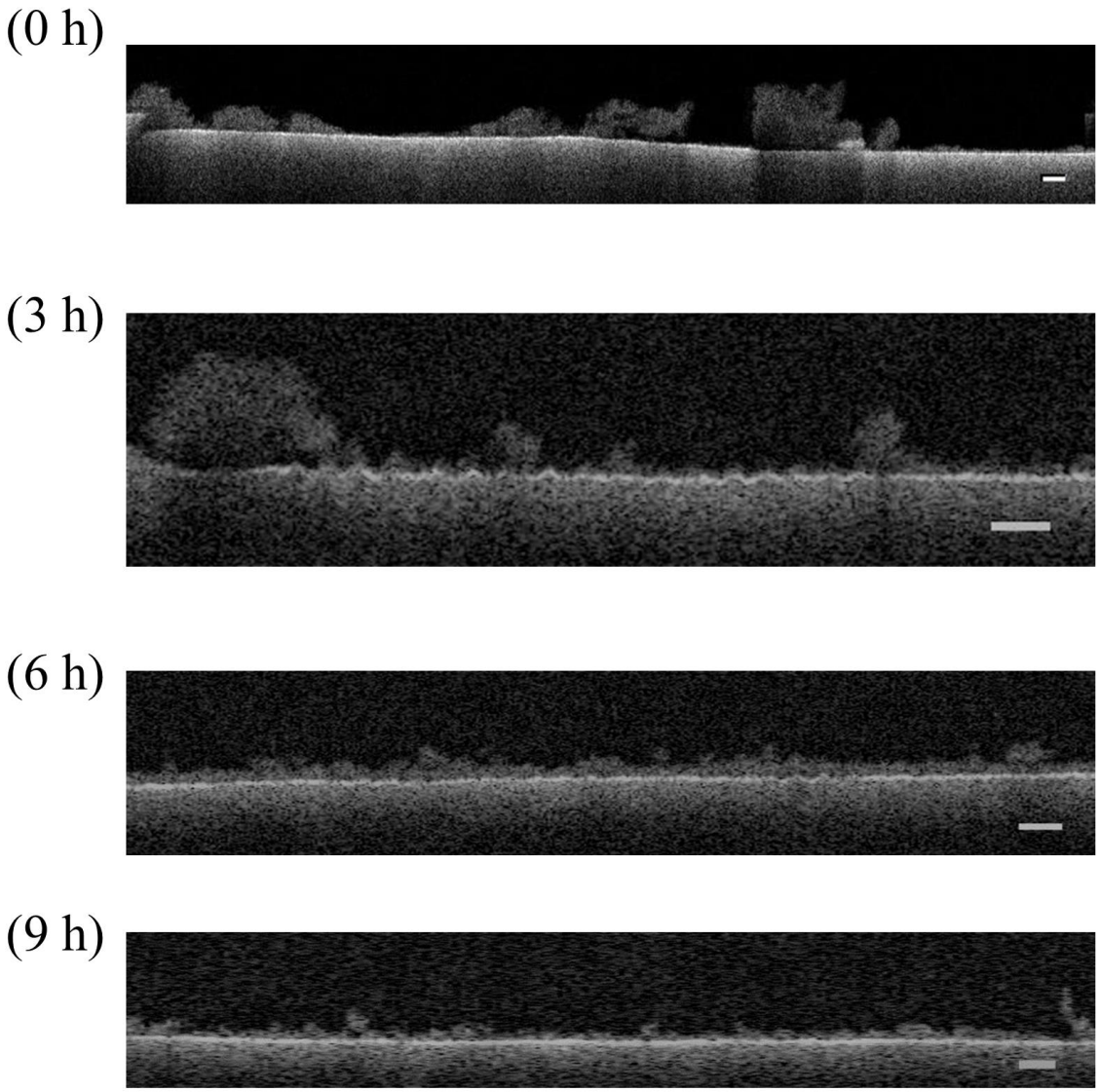

Figure S6. Photocatalytic eradication kinetics of *S. epidermidis* biofilms from the g-C_3N_4 coupon surface characterized by optical coherence tomography (OCT). Mature biofilms were first grown on the g-C_3N_4 coupon surface in 10-fold diluted TSB in the dark for 3 days prior to visible white LED light exposure (0 h), and next the mature biofilms were treated under LED light irradiation for different durations (3, 6, and 9 h) in 10-fold diluted TSB. Scale bars are 100 μm.

References

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