Supporting Information

Strategy on Antimicrobial Regulation Based on Fluorescent Conjugated Oligomer-DNA Hybrid Hydrogel

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Experimental Section:

***Materials and Instruments*:** All Chemicals were of analytical grade and were used as received. All aqueous solutions were prepared with ultrapure water purified using a Millipore filtration. OPE (Oligo (phenylene ethynylene)) was synthesized according to the reported procedure in the literature.[1] All bacterial work was accomplished under aseptic conditions. The Gram-negative (Escherichia coli) bacteria was purchased from Tiangen Co. Salmon sperm DNA and ethidium bromide (EB) was purchased from Sigma. The 1H NMR spectra was recorded on a Bruker Avance 300 MHz spectrometer. The OD of the *E.coli* cells suspension was regulated on a Jasco V-550 spectrometer. The photographs were obtained by Canon digital camera. Images

of scanning electron microscopy (SEM) were taken on Hitachi S-4300 scanning electron microscopy. Phase contrast bright-field and fluorescence images were taken with a fluorescence microscope (Olympus 1 × 71) with a mercury lamp (100 W) as light source. The excitation wavelengths were D455/70 nm and D380/30 nm for EB and OPE, respectively. Experiments for photosensitized damage of bacteria were performed with a metal halogen lamp (Mejiro Genossen MVL-210) with a wavelength between 400 and 800 nm. The light dose used for irradiation was 162 J • cm^{-2} and the intensities of incident beams were checked by a radiometer. The experiments were carried out in 0.9 % Sodium chloride solution.

1. Synthesis of DNA-OPE hydrogel

First, salmon DNA (30.0 mg, 45.45 μmol base pair) was dissolved in 2 mL HEPES buffer (pH = 7.0); DNA hydrogel was made by cross-linking with 17.0 μL ethylene glycol diglycidyl ether (1%). The DNA concentration at cross-linking was 1.5% (w/w). Then, OPE solution (10 mL, 80 μg/mL) was added into the DNA hydrogel. The DNA-OPE hydrogel (the ratio of positive charge and negative charge in the DNA-OPE hydrogel is about 1:90) was gained after the hydrogel solution was dialyzed against distilled water for 24 h.

2. Characterization of DNA hydrogel

Optical properties and morphologies of DNA-OPE hydrogel

The optical properties and morphologies of DNA-OPE hydrogel were characterized with scanning electron microscopy (SEM) and fluorescence microscope (Figure 1).

The experimental details are shown below:

SEM measurements

To gain more insight on the morphology of the GEL, SEM characterization was included in this study. The GEL was added to clean silicon slices and the specimen was Freeze-dried for 3 h. Finally, the sample was coated with platinum before examination in SEM. Then the images of scanning electron microscopy (SEM) were taken on Hitachi S-4300 scanning electron microscopy.

Fluorescent microscope measurements

In order to gain a visual display on the optical properties of DNA-OPE hydrogel and the toxicity of the GEL towards *E.coli* in the presence and absence of DNase, Fluorescent microscope was used to characterize them in this study. After the above-mentioned antibacterial experiments, EB was added to the samples. Then, 50 μL *E.coli* suspensions with EB were dropped onto clean glass slides by Eppendorf pipette. Cover-slips were covered before examination in fluorescent microscope. Fluorescent microscope specimens of DNA and GEL were gained by dropping the corresponding solution onto clean glass slides, covering the cover-slips and then Freeze-dried for 3 h .The fluorescence images were obtained with a fluorescence microscopy at 2000 ms exposure time for *E.coli* suspensions and 100 ms for the GEL. The false color of EB is red and the type of light filter is D455/70 nm exciter, 500 nm beamsplitter, and D600/50 nm emitter. Magnification of object lens is 40×. The false color of OPE is blue and the type of light filter is D380/30 nm exciter, 420 nm

beamsplitter, and D460/50 nm emitter. Magnification of object lens is 40×.

The demonstration of hydrolysis of the DNA-OPE hydrogel

14 μL Sterile water, 2 μL 10 × DNase reaction buffer (400 mM Tris-HCl, 80 mM $MgCl_2$, 50 mM DTT, pH =7.5), 1 μL GEL solution (2 mg/mL) and 3 μL DNase(1 U/μL) were added successively. After hydrolyzing for 30 min, 5 μL 6 × Loading buffer was added. Then 8 μL of the above-mentioned solution was added into the agarose gel for electrophoresis. The rest of the hydrolyzate solutions were used to obtain fluorescence microscopy images according to the above-mentioned procedures. The fluorescence images were taken via fluorescence microscopy at exposure time of 5000 ms for hydrolyzates and 100ms for GEL. The false color of OPE is blue and the type of light filter is D380/30 nm exciter, 420 nm beamsplitter, and D460/50 nm emitter. Magnification of object lens is 40×.

3. Regulating of Antibacterial Experiments.

A single colony of *E. coli* on a solid Luria-Bertani (LB) agar plate was transferred into 5 mL of liquid LB culture medium in the presence of 50 μg/mL Kanamycin and was grown at 37 ^{o}C for 12 h. Bacteria were gained by centrifuging (7100 rpm for 2 min) and washing by 0.9 % sodium chloride solution for three times. After discarding the supernatant, the remaining *E.coli* was resuspended in 0.9 % sodium chloride solution. The suspension was diluted until its OD was 1.0 at 600nm. The phototoxicity of GEL, and its hydrolyzate [6 μL 10×DNase reaction buffer (400 mM Tris-HCl, 80 mM $MgCl_2$, 50 mM DTT, pH = 7.5), 50 μL GEL solution (2 mg/mL), 10

μL DNase(1 U/μL)] were added successively for hydrolyzing for 30 min. After incubated in the dark at room temperature for 20 min, The *E.coli* cells suspensions were exposed to an optical fiber of 90 mW · cm^{-2} white light for 30 min (162 J · cm^{-2}). Then the cell suspensions were serially diluted 6 × 10^4-fold in 0.9 % sodium chloride solution and a 100 μL portion of the diluted *E. coli* was spread on the solid LB agar plate, The colonies formed units (CFU) were counted after 12-14 h incubation at 37 ^oC. The reduction fraction was determined by the following Formula.

$$\%\ \text{CFU Reduction} = 1 - \frac{\text{CFU of the samples incubated with the GEL or hydrolyzates}}{\text{CFU of the control that carried out in the absence of GEL}}$$

The diameter of the solid agar plates was 90 mm. Bacterial death assays were also carried out by ethidium bromide (EB) staining. After EB was added to the samples that had been illuminated by white light, the samples were then observed by fluorescence microscopy.

Supplementary Figure:

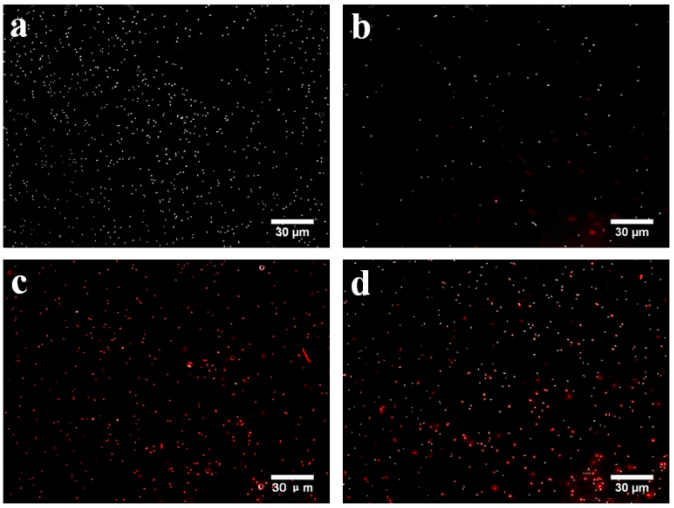

Fig. S1. Antibacterial activities of GEL and GEL/DNase towards Gram-negative bacteria *E. coli*: Overlapping images of *E. coli* suspensions under the phase contrast bright-field and under the fluorescence field incubated with (a) blank (without photosensitizer), (b) GEL, or (c) GEL/DNase under irradiation and (d) GEL/DNase, [GEL]=0.2 mg/mL. Bright cells indicate live bacteria, and red staining indicates dead bacteria.

Reference:

[1] Z. Zhou, T. S. Corbitt, A. Parthasarathy, Y. Tang, L. K. Ista, K. S. Schanze and D. G. Whitten, *J. Phys. Chem. Lett.*, 2010, **1**, 3207-3212.