Supporting Information

Development of an Observation Platform for Bacterial Activity Using Polypyrrole Films Doped with Bacteria

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Experimental

Chemicals. Monomer, 3,4-ethylenedioxythiophene (EDOT) was purchased from Sigma-Aldrich. Pyrrole, potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), and sodium chloride (NaCl) were obtained from Wako Pure Chemical Industries (Japan). Alginic acid was purchased from MP Biomedicals (France).

Nutrient Broth (NB) and tryptone soya broth (SCD broth) were obtained from Eiken Chemicals (Japan). The bacterial viability kit, LIVE/DEAD BacLight L7007, was purchased from Molecular Probes. Bacterial samples of *Pseudomonas aeruginosa*, *Escherichia coli* (O Rough:H48), *Acinetobacter calcoaceticus*, *Serratia marcescens*, and *Bacillus subtilis* were acquired from the Biological Resource Center (NBRC, Japan) and *Shewanella oneidensis* MR-1 was obtained from American Type Culture Collection (ATCC). Ultrapure water (resistance >18 MΩ) was used throughout this work.

Bacterial cultivation. *P. aeruginosa*, *E. coli*, *S. oneidensis*, *A. calcoaceticus*, and *S. marcescens* were cultured in the NB broth, while *B. subtilis* was grown in the SCD broth. Both were incubated overnight at 30°C. The bacterial suspensions were centrifuged at 2000 g for 10 min and the supernatant was discarded. The bacterial pellet was then resuspended in 0.85% NaCl (saline). This isolation procedure was repeated twice to obtain a purified bacterial target.[1–4]

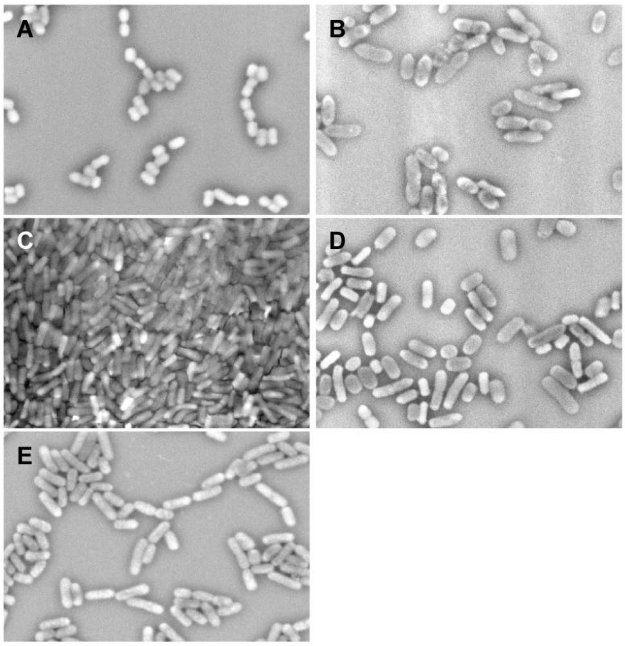

Figure S1. SEM images of (A) *Acinetobacter calcoaceticus*, (B) *Serratia marcescens*, (C) *Bacillus subtilis*, (D) *Escherichia coli*, and (E) *Pseudomonas aeruginosa*.

Electrochemical experiments. All the microbial experiments were performed under strictly sterile conditions. All electrochemical experiments were performed with a potentiostat (model 842B, ALS) equipped with quartz crystal microbalance (QCM, SEIKO EG&G., Japan).

Preparation of bacteria-doped PPy. An Ag | AgCl | saturated KCl || electrode and platinum wire electrode served as reference and counter electrodes, respectively. Unless otherwise noted, a film of PPy doped with *P. aeruginosa* (PPy/*P. aeruginosa*) was prepared as follows: pyrrole (5 μmol) and 0.50 mL of phosphate buffer (pH 2.5–5.3) were added to the cell precipitate obtained above. This dispersion had a pyrrole concentration of 10 mM and a *P. aeruginosa* density of 1.5 × 10^9 CFU mL^{-1}.[1–3] To determine the effect of alginate doping in the PPy, a polymerization mixture with 0.33% alginate was prepared. These dispersions were oxidized at 0.98 V against the Ag | AgCl electrode for 300 s to deposit a PPy film on a QCM gold electrode (Seiko EG&G; surface area, 0.2 cm^2), which had been pretreated by soft plasma etching (SEDE-GE, Meiwa Fosis, Japan) for 30 s. The PPy, thus deposited, gave the film a thickness of a few micrometers.

Preparation of bacteria-doped PEDOT. A sheet of indium tin oxide (ITO)-coated glass (resistance: 10 Ω) was cut into a 0.5 × 1.3-cm strip, sonicated in ethanol for 30 min, and then coated with an adhesive polypropylene tape (Scotch® Filament Tape 898), which had a hole (diameter, 4 mm) for use as the working electrode. The Ag|AgCl electrode and a platinum mesh (0.7 × 1.5 cm) were employed as the reference and counter electrodes, respectively. The electrodes were placed in a glass cell, which contained 2 mL of aqueous solution consisting of 10 mM EDOT and purified bacteria (2.6 × 10^7 cells mL^{-1}) in a pH 5.3 phosphate buffer. A constant potential of 1.05 V was applied for 100 s to deposit a PEDOT on the ITO electrode. Freshly polymerized PEDOT/bacteria films were then rinsed with an ample amount of water and immediately subjected to microscopic analyses.

Thin-layer-cell voltammetry. To monitor the bacterial activity, a thin-layer electrochemical cell was employed. Polypyrrole films doped with *E. coli* were deposited on ITO glass, which had a working electrode area of 4 mm in diameter and was prepared as described above. After the PPy deposition, the electrode was rinsed with water and 700 μL of phosphate buffer (pH 7.0) was applied only on this area. Afterwards, a piece of filter paper (No. 1, diameter 55 mm, Advantec, Japan), folded in half, was placed on the ITO glass strip. Another ITO glass, used as the counter electrode, was placed on the filter paper, with about 5 mm of the paper protruding from the glass sandwich. This cell assembly was fixed and bound with an adhesive Teflon tape. A

reference electrode tip (approximately 1 mm in diameter) was inserted in between the two layers of the protruding filter paper to make a stable electrical contact. Between voltammetric examinations, the cell was disconnected from the potentiostat to minimize oxygen consumption at the working electrode and oxygen generation at the counter electrodes, both of which occurred in parallel with oxygen consumption due to bacteria activity. All the thin layer cell experiments were performed at 37°C in a thermostated Faraday cage.

Fluorescence microscopic observation. The bacteria doped CPs film was stained with fluorescence pigments, SYTO9 and propidium iodide (PI), according to the manufacturer's instructions for the Bacterial Viability Kit (Molecular Probes). Bacteria immobilized in the polymer films were observed by fluorescent microscope (BX51, Olympus Co.; Japan). [1–3]

Scanning electron microscopic observations. The surfaces of the polymer films were also imaged by scanning electron microscopy (SEM; TM-1000, Hitachi; Japan).[1–3] SEM images of the labeled bacteria were obtained after adding 10 μL of a suspension to a conducting Si (111) wafer (*p*-type) and drying in atmospheric air.[4]

SEM observation for polypyrrole film-formation. Figure S2 (A) through (C) shows the SEM images of *P. aeruginosa* during PPy polymerization at pH 2.5.[1–3] The bacteria were immobilised in a self-standing fashion even at the very first moment of the polymerization (30 s), at which a polymer film was scarcely seen. More bacteria were fixed on the surface at 40 s, and their bottom parts were clearly embedded in the film within 60 s.

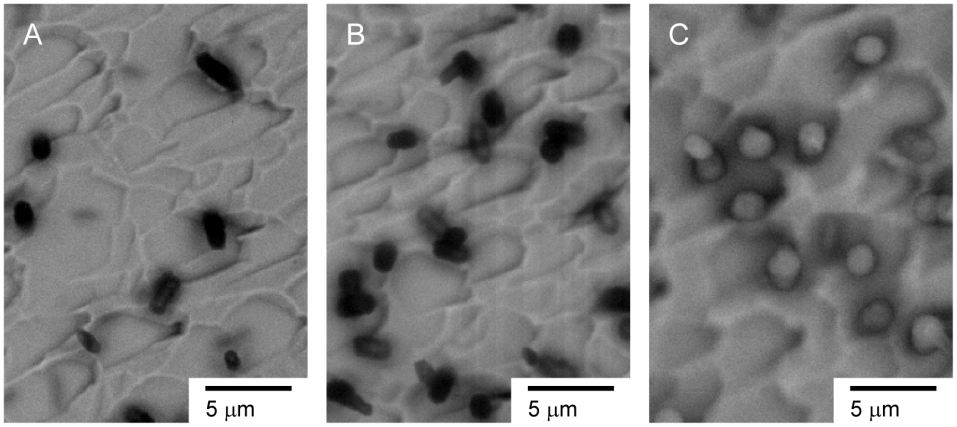

Figure S2. SEM images of PPy/*P. aeruginosa* in the course of polymerization at pH 2.5. Polymerization period, (A) 30 s, (B) 40 s, and (C) 60 s.

References

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