Electronic Supplementary Information

Spontaneous and specific binding of enterohemorrhagic *Escherichia coli* to overoxidized polypyrrole-coated microspheres

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Experimental

Chemicals. All chemicals were reagent grade. Ultrapure water (> 18 MΩ cm) sterilized with UV light was used in all experiments. For safety reasons, experiments were conducted with genetically modified verotoxin-nonproducing *Escherichia coli* PV856 (O157:H7), *E. coli* PV276 (O157:HNM), *E. coli* PV01-198 (O26:H11), and *E. coli* PV03-017 (O26:HNM). These strains were provided by Prof. M. Miyake, Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, and by Dr. K. Seto, Osaka Prefectural Institute of Public Health. *E. coli* K-12 (O Rough:H48, NBRC3301), *P. aeruginosa*, *S. marcescens*, and *A. calcoaceticus* were purchased from National Institute of Technology and Evaluation Biological Resource Center (NBRC).

SYTO9® and Hoechst 33258 (5'-bi-1H-benzimidazole trihydrochloride) were used as fluorescent dyes. Nafion®117 was purchased from Sigma-Aldrich, while gold-coated microbeads were purchased from GreenChem. Inc.[1]

Bacterial culture. Bacterial cultures and experiments were executed in a biosafety level 2 laboratory designed and managed in accordance with safety regulations. Liquid cultures were grown at 303 K for 18 h in E-MC35 agar broth (Eiken Chemical Co. Japan). Similarly, colonies were suspended in 30 mL E-MC35, and cultured at 303 K for 18 h. Cells were then harvested by centrifugation at 7,000 *g* for 15 min and washed four times for 1 min each in fresh phosphate buffer.

Apparatus. Samples were imaged on a TM3030 (Hitachi, Japan) scanning electron microscope operating at accelerating voltage 5 kV, and on a BX51 fluorescent microscope (Olympus, Japan). Fluorescent spectra were collected on FP-6300 (Jasco, Japan).

Fabrication of microspheres imprinted with bacterial cells. Gold-coated microbeads (5 mg), the surface of which was modified with a self-assembled aminothiophenol monolayer, were dispersed in 20 mL 25 vol% aqueous ethanol containing 1-50 µL 20 % nafion. After ultrasonication for 20 min, the dispersion was immediately mixed with 30 µL pyrrole and 5 mL *E. coli* O157:H7 (3.8×10^9 cells mL^{-1}), and stirred for 40 min. Subsequently, polymerization was induced by oxidation with $(NH_4)_2S_2O_8$ for 12 hours at room temperature. Polymer-coated microspheres were then collected by centrifugation at 1,500 *g*, and washed with ultrapure water. To remove bacterial cells, microspheres were redispersed in aqueous 0.1 M NaOH, stirred for 3 hours, washed with ultrapure water, and dried in a vacuum chamber.

Dark-field microscopy. In dark-field microscopy, scattered light is detected, while directly transmitted light is blocked with a dark-field condenser. Samples were thus imaged using an Eclipse 8oi optical microscope (Nikon, Japan) equipped with a dark-field condenser, a 100 W halogen lamp, and a charge-coupled device camera. Light-scattering spectra were obtained using a USB4000 miniature grating spectrometer (Ocean Optics) coupled to the microscope via an optical fiber with core diameter 400 μm.[2] Typical acquisition times were 400 ms. Spectra were corrected for spectral variations in system response, and white-light intensity distribution (main intensity 600 nm) through division by bright-field spectra was recorded through the sample. The collection volume was nearly diffraction-limited for the 100× objective (NA 0.9)/fiber combination used, with cross-sectional area approximately ~10 μm^2. Samples were prepared by mounting on a glass slide 5 μL microspheres, bacterial suspensions, and mixtures thereof, and air drying for 1 hour.

Cell counting. Microspheres imprinted with *E. coli* O157:H7 (2.0 mg) were incubated with 2.0×10^7 cells mL^{-1} of SYTO9-stained *E. coli* O157:H7, O157:HNM, O26:H11, O26:HNM, O rough, *P. aeruginosa*, *S. marcescens*, and *A. calcoaceticus*. After incubation for 3 hours, 5 μL of the supernatant cleared by gravity was mounted on a glass slide, and imaged on a fluorescent microscope to count the number of cells remaining in the presence (n) or absence of microspheres (n_0).

Results

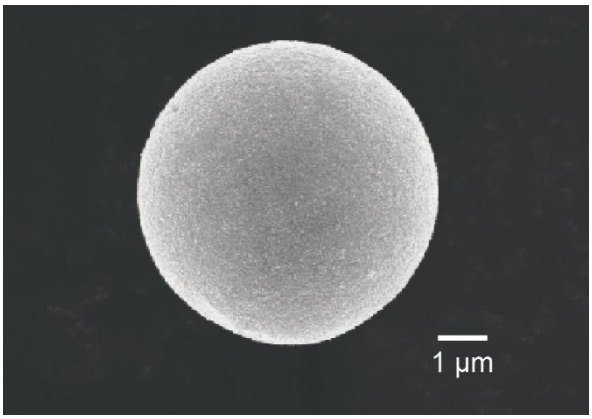

Fig. S1 SEM image of gold-coated acrylic resin microsphere.

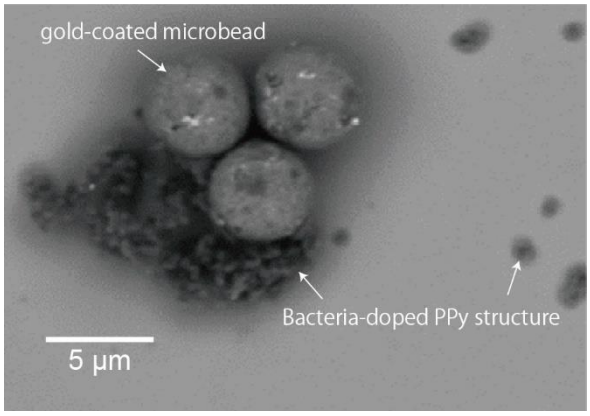

Fig. S2 SEM image of microspheres prepared without nafion.

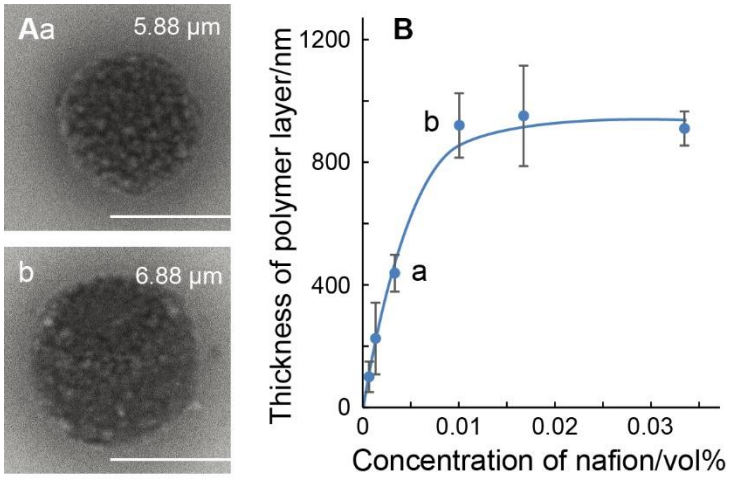

Fig. S3 (A) SEM images of microspheres prepared with (a) 0.0030 and (b) 0.010 vol% nafion. Average diameters are indicated (n = 10). The scale bars in SEM images are 5 μm. (B) Thickness of the polymer complex without cells as a function of nafion concentration.

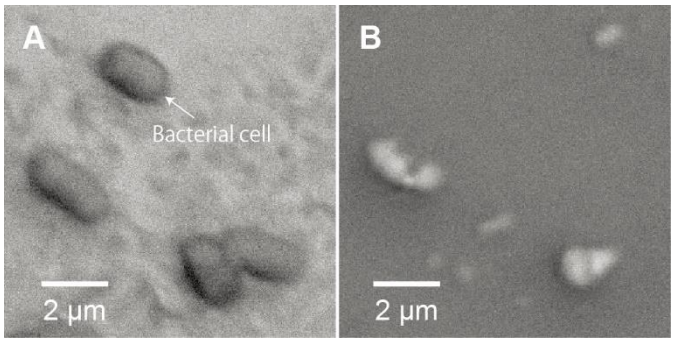

Fig. S4 SEM images of *E. coli* O157:H7 (A) before and (B) after incubation for 3 h in an aqueous 0.10 M NaOH.

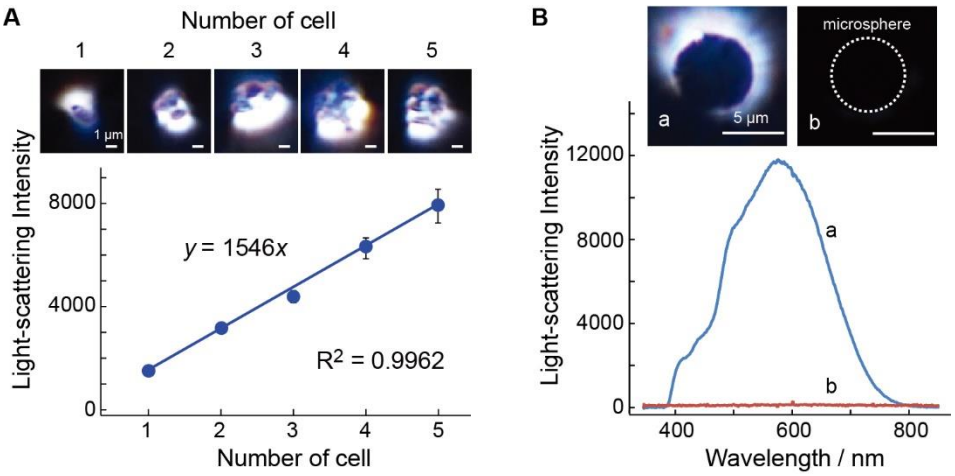

Fig. S5 (A) Dark-field images and light-scattering intensity of *E.coli* O157:H7 cells at different aggregation numbers. The scale bars in dark-field images are 1 μm (n = 10). (B) Dark-field images and light-scattering spectra of a single microsphere (a) before and (b) after doped bacteria are removed. The scale bars in dark-field images are 5 μm (n = 10).

References

1. (a) H. Shiigi, S. Shirai, T. Fujita, H. Morishita, Y. Yamamoto, T. Nishino, S. Tokonami, H. Nakao and T. Nagaoka, *J. Electrochem. Soc.*, 2013, **160**, H630; (b) S. Tokonami, Y. Yamamoto, Y. Mizutani, I. Ota, H. Shiigi, T. Nagaoka, *J. Electrochem. Soc.*, 2009, **156**, D558; (c) Y. Yamamoto, S. Takeda, H. Shiigi, T. Nagaoka, *J. Electrochem. Soc.*, 2007, **154**, D462.
2. (a) H. Shiigi, T. Fujita, X. Shan, M. Terabe, A. Mihashi, Y. Yamamoto, T. Nagaoka, *Anal. Sci.*, 2016, **32**, 281; (b) H. Shiigi, S. Kimura, T. Fujita and T. Nagaoka, *Anal. Sci.*, 2015, **31**, 577.