Supplementary Information

Engineering antimicrobial coating of archaeal poly-γ-glutamate-based materials using non-covalent crosslinkages

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Supplementary Figure 1. (a) $^{13}$C NMR and (b) $^1$H NMR spectra of authentic DEQ$^{2+}$; the insets illustrate assigned DEQ$^{2+}$ structures.
Supplementary Figure 2. WAXS spectra of PGAIC-precursor standards. Authentic samples (a), poly-γ-glutamate (PGA; from Wako, Japan); and (b) dequalinium di-chlorides (DEQ\textsuperscript{2+}; from Sigma Co., USA).
Supplementary Figure 3. Electron microscopy of PGAIC-coated microfibers on plastic surfaces.

SEM images, from (a) a non-coated HIYEX non-woven plastic cloth (or sheet) (from Kuraray, Japan); the PGA/HDP-coated sheets (b) before and (e) after the EtOH (> 99.5 wt%)-soaking process (see Fig. 5); and the PGA/DEQ-coated sheets (d) before and (e) after the same severe treatment. The length of the black bar is 10 μm. In particular, the image e indicates the excellent durability of the PGA/DEQ coatings against alcohols.
Supplementary Figure 4. Growth curves of *E. coli*.
The viable cells (~1.7 × 10^5 CFU) were first inoculated into Luria–Bertani (LB) media (5 mL), each carrying a disk (12 mm dia.) formed from PGA/DEQ-coated sheets (*open symbols*) and PGA/HDP-coated sheets (*closed symbols*) treated in the following ways: (a) soaking in EtOH (*circles*) or CHCl₃ (*triangles*); and (b) soaking in 1.5% NaCl (*diamonds*), 3.0% NaCl (*squares*), or 5.0% NaCl (*squares with crosses* (or *ballet boxes with an x*)). The (net) growth rates of the colonies were then estimated by monitoring the culture turbidity at 600 nm using a spectrophotometer (*n* = 3). The standard deviations observed in the latter treatment were actually <5% (0 to a maximum of 0.04). Symbols in parentheses represent the images of the cultures (*top*) acquired at the end of 36-h cultivation; the BPB-stained disks (*bottom*) were essentially the same as the PGAIC-coated sheets used in the experiments, the darkness of which briefly corresponded to the quantity of PGAICs retained on the surfaces.
Supplementary Figure 5. pH-response tests of PGA/DEQ coatings.
Panel a: Contrary to a general view that PGAIC (e.g., PGA/HDP) coatings are labile following soaking in the universal buffer “Carmody” (○) composed of borate, citrate, and phosphate\(^1\)\(^2\), PGA/DEQ exhibited increased resistance to the same buffer (pH 3–12), except in the range pH 5–6, in which citrate molecules are mainly (theoretically) transformed into the di-sodium form. b Further experiments using a combination set (each 0.1 M) comprising several buffers [glycine-HCl (●; pH 2.5–3.5); Na citrate (○; 3.5–5.5); Na acetate (▲; 3.5–5.5), MES-NaOH (△; 5.5–6.5); MOPS-NAOH (◆; 6.5–7.5); HEPES-NaOH (◇; 7.5–9.0); glycine-KOH (■; 9.0–11.0); and Na\(_2\)HPO\(_4\)-NaOH (☐; 11.0–12.0)]\(^3\) also suggested that citrate had a peculiar effect on the extremely stable PGAIC antimicrobials. Retained antimicrobial performance was assessed by comparing log-reduction scores \((n=5)\) in the presence of PGA/DEQ-coated disks after soaking with the indicated buffers. In the present experiment, all the estimated standard deviation scores were actually <5\% (0 to a maximum of 0.26). These imply that the removability of PGA/DEQ coatings, which are extraordinary durable over various pH ranges, can be controlled by adjusting the proportion of certain buffer components.
Supplementary Figure 6. Potent antimicrobial performance of PGAIC coatings.

*E. coli* cells (≈5.5 × 10⁵ CFU) were inoculated into LB media (5 mL), each carrying a disk (12 mm *dia.*; 0.35 mm *thick*) from the non-coated (*crosses*), and PGA/DEQ- (*circles*) and PGA/HDP-coated (*triangles*) sheets before (*open symbols*) and after (*closed symbols*) the severe treatment using EtOH (*see Fig. 5*).

(a) Viable cell counts in the liquid culture media (*n=3*), indicating the expression of a fast elimination (or killing) mechanism (within 10 min), followed by sustainable antimicrobial performance (after 180 min). Particularly, it is noteworthy to be significant in the durable (*e.g.*, extraordinary water-resistant) PGA/DEQ coatings from the viewpoint of improved contact-killing surfaces⁴. (b) Counts of viable cells adhered in the disk samples after cultivation (*n=3*). The moisture of samples was gently drained, and their weights were calculated to be 28 mg averagely (*n=15*), the scores of which were virtually constant regardless of the incubation times, presumably owing to the size stability of HIYEX non-woven plastic cloth. Each drained sample was then soaked into 1 mL of 100 mM citrate *di*-salts at 25°C for 10 min, and the resulting suspensions were subjected to the counting experiment of viable cells (*see the Method section*). On the PGA/DEQ-coated disks, the viable cells (*though* their numbers are surely not large) were counted even under the circumstances where *E. coli* cells have disappeared from the liquid media (*e.g.*, after the 540-min incubation), providing insight into a functional surface actively involved in bacteria elimination.
Supplementary Figure 7. Sustainable antimicrobial performance of PGAIC coatings.

Abbreviations: E. c, Escherichia coli; S. a, Staphylococcus aureus; B. s, Bacillus subtilis. Cells of microorganisms (~5.5 × 10^5 CFU) were inoculated into LB media (5 mL), each carrying a disk (12 mm dia.) from the non-coated (a), PGA/HDP-coated (b, before; c, after the EtOH soaking), and PGA/DEQ-coated (d, before; e, after the EtOH soaking) (see Fig. 5), and then cultured at 37°C for 5 days. The cultures of images a and c actually reached to their stationary phase after 24-h incubation, whereas the use of PGA/DEQ coatings (images d and e) brought about the long-term suppression against cell growth of Gram-positive bacteria (e.g., S. a and B. s) in addition to Gram-negative bacteria (e.g., E. c).
Supplementary Figure 8. Schematic representing a possible novel microbicidal mechanism called “Capture–Killing”.

Steps (a), access and capture of microbial cells (or infectious particles); (b), highly reliable attack on the captured targets by released drugs; and (c), chemical disruption of the captured targets.
Supplementary Figure 9. Kinetics of PGAIC formation.

The initial concentration (mg/mL) of PGA is 2.5±0.1, indicating the presence of carboxyl residues at ~20 mM. Panel a: the dose-dependency of PGAIC (i.e. PGA/DEQ and PGA/HDP) formation against the QA-type surfactants used (i.e., DEQ<sup>2+</sup> and HDP<sup>+</sup>). The increase in turbidity of the reaction mixtures implied the accumulation of water-insoluble PGAICs. b Sigmoid-fitting (thus non-hyperbolic) events in the formation of PGAICs (○, PGA/DEQ; ●, PGA/HDP). In the kinetic analysis, the non-ideal competitive adsorption (NICA) model<sup>5–7</sup> (or the Hill equation in enzymology) prefers to the Langmuir model (or the Michaels–Menten equation). c Cooperative PGAIC formation was first demonstrated and then kinetically characterized using the NICA model. The cooperativity (n)/affinity (K<sub>d</sub>, mM) scores of PGA for DEQ<sup>2+</sup> and HDP<sup>+</sup> can be found in the table-type inset. Interestingly, the composition analysis using NMR proved that the carboxyl groups of all the PGAICs in a were constantly and completely transformed with QA moieties, presumably owing to their (potent) cooperative bindings.
Supplementary Figure 10. Schematic diagrams of (A) the *onsite* synthesis of the PGA/DEQ coatings and (B) their quantitative colorimetric assay. 

Steps (a), first coating of a PGA solution on the surfaces of base materials; (b), surface functionalization *via* the spontaneous coating of PGA as a widely applicable adhesive; (c), second coating with a DEQ$^{2+}$ solution on the PGA-mounting surfaces to briefly form PGA/DEQ *onsite*; (d), 30-min soaking in methanol (1 mL/disk; repeated a total of three times per treatment process) with gently shaking to wash out excess (unbound) DEQ$^{2+}$ and to leave only durable PGA/DEQ coatings *onsite*; (e), 10-min immersion of PGA/DEQ-coated materials in a BPB concentration (1 mL/disk) to form BPB/DEQs (*see* Supplementary Fig. 11, panel a); (f), 5-min soaking in water (5 mL/disk; repeated five times) to remove unbound BPB anions and remain water-insoluble BPBICs; (g), 24-h soaking in methanol (1 mL/disk) to extract BPBIC molecules from the dried surfaces of the resulting disks and ultimately determine the amount of PGAICs thereby immobilized as PGA/DEQ coatings; and (h), quantitative analysis of BPBICs (*see* Supplementary Fig. 12).
Supplementary Figure 11. Formation of water-insoluble complexes (BPBICs) comprising BPB anions and cationic surfactants on PGAIC-coated surfaces. Predicted structures (a), a DEQ-bound form (*namely* BPB/DEQ); and (b), an HDP-bound form (BPB/HDP). The inset images depict the solvation of each BPBIC in water (*top*) and methanol (*bottom*).
**Supplementary Figure 12. Spectrophotometry of BPBICs.**

Left panel, (a) absorption spectra in methanol of BPB/DEQ (*solid line*, red), BPB/HDP (blue), and free BPB (*dotted line*, black); the inset depicts free DEQ (red) and HDP (blue). The specific absorption of BPB/DEQ is at 330–350 nm (rose-pink zone) and that of BPB/HDP is at 410–450 nm (light-blue zone); however, the maximum absorption wavelength is commonly around 590 nm (yellow zone). Right panels, the calibration curves of (b) BPB/DEQ and (c) BPB/HDP solutions. The insets illustrate the BPB-binding models of BPBIC; hence, a DEQ molecule (*top*) can capture twice the amount of dye as a HDP molecule (*bottom*).
| Microorganisms               | Found concentrations (ppm)\(^a\) |
|-----------------------------|----------------------------------|
|                             | DEQ\(^{2+}\) | HDP\(^+\) |
| *Staphylococcus aureus*     | 4             | 2          |
| *Escherichia coli*          | 32            | 64         |
| *Pseudomonas aeruginosa*    | 100           | >500       |
| *Candida albicans*          | 8             | 25         |
| *Aspergillus niger*         | 16            | 300        |

Supplementary Table 1. Minimal inhibition concentrations (MICs) of DEQ\(^{2+}\) and HDP\(^+\). \(^a\)The values were determined according to the guidelines provided by the Clinical and Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards)\(^9\).
Supplementary references

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