Supporting Information for:

Bacteria Poration on Modified Boron-Doped Diamond Electrode Surfaces Induced by Divalent Cation Chelation

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Supporting Information: including experimental details, 9 pages, 4 Figures.

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Electrode modification process

The EDC/NHS method was applied to crosslink amide with carboxylic acids.[1,2] The mechanism of EDC/NHS reaction is shown in **Figure S-1.**[1] The mechanism involves the mixing of glycolic acid (1) with 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (2). The O-acylisourea intermediate (3) was formed by reaction between the carboxylic acid and EDC, which is usually unstable in aqueous solutions. NHS (4) was added for stabilizing the final product and improving the crosslink efficiency. The NHS reacts with the O-acylisourea intermediate and forms the semi-stable amine reactive NHS ester (5). Compound (5) crosslinks with the amino group on the APTES-BDD/OTE (6). The final N-propyl-2-hydroxyacetamide functional group was formed on the BDD/OTE (7). The process was conducted in the pH 5.0 MES buffer, which is suitable for carbodiimide reaction without primary amine or carboxyl groups present.

These reaction steps were carried out on an APTES-BDD/OTE. First, 5% volume of GA was dissolved in the 5 mM MES buffer solution in the beaker and stirred for 30 minutes until chemicals were well dissolved. 5 mM EDC was added into this solution to react with GA for another 30 minutes. Then, 5 mM water soluble NHS and APTES-BDD/OTE electrode were added into the beaker for further crosslinking process of GA on the APTES-BDD/OTE. The beaker was covered with paraffin and set in the shaker with a rotation speed of 160 rpm at room temperature for 20 hours. Last, the OH-BDD/OTE was washed by water three times to remove the unreacted GA and dried at room temperature.

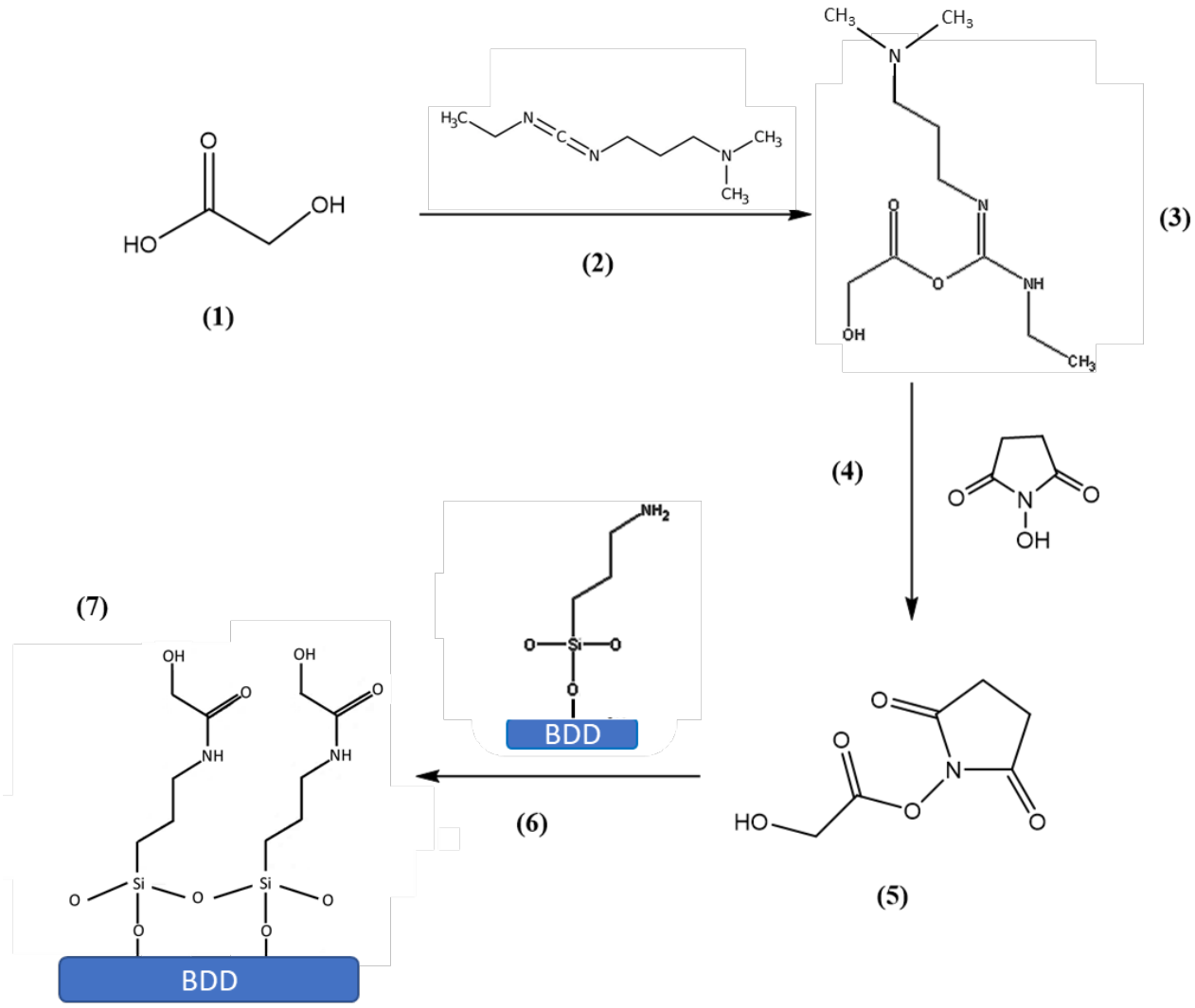

Figure S1. The mechanism of EDC/NHS reaction (1) glycolic acid, (2) EDC, (3) unstable reactive O-acylisourea ester, (4) NHS, (5) glycolic acid-NHS (6) APTES (7) N-propyl-2-hydroxyacetamide functional groups.

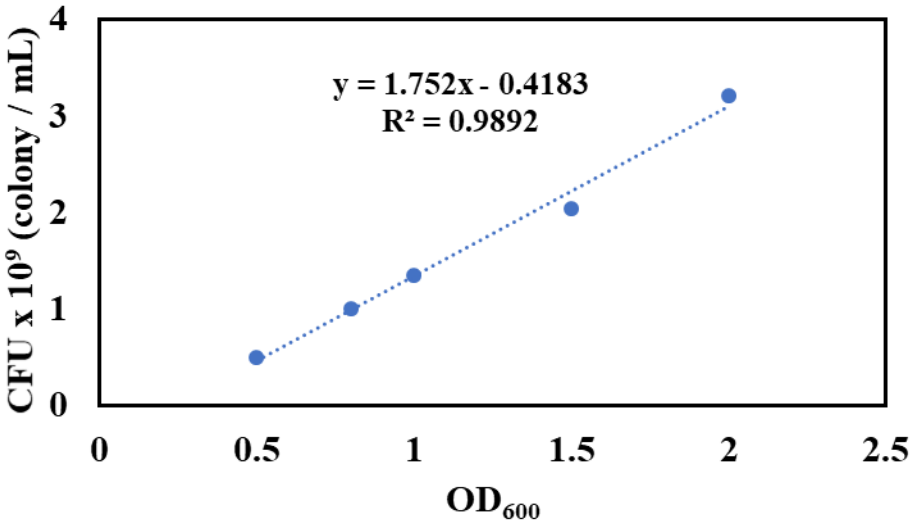

Figure S2. The relationship between the reading number of OD_{600} and bacteria number in 1 mL solution.

Surface modification coverage calculation

$(C:N)_{OH\text{-}BDD/OTE} = (1\text{-}X)\ (C:N)_{BDD/OTE} + X(C:N)_{APTES+GA}$

$(C:Si)_{OH\text{-}BDD/OTE} = (1\text{-}X)\ (C:Si)_{BDD/OTE} + X(C:Si)_{APTES+GA}$

Definition :

$(C:Si)_{OH\text{-}BDD/OTE}$: The Carbon and Silicon ratio on the OH-BDD/OTE based on XPS results.

$(C:Si)_{BDD/OTE}$: The Carbon and Silicon ratio on the BDD/OTE based on XPS results.

$(C:Si)_{APTES+GA:}$ The Carbon and Silicon ratio based on the chemical structure on the BDD/OTE.

For example:

Assuming one APTES compound binds with one -OH group on the BDD. Therefore, the C/Si ratio for $(C:Si)_{APTES+GA}$ is 3. The C/Si ratio on BDD/OTE and OH/BDD-OTE were measured as 64.5 and 17.4, respectively. The equation can be written as $64.5(1\text{-}X)+3X = 17.4$ and the coverage can be calculated from X.

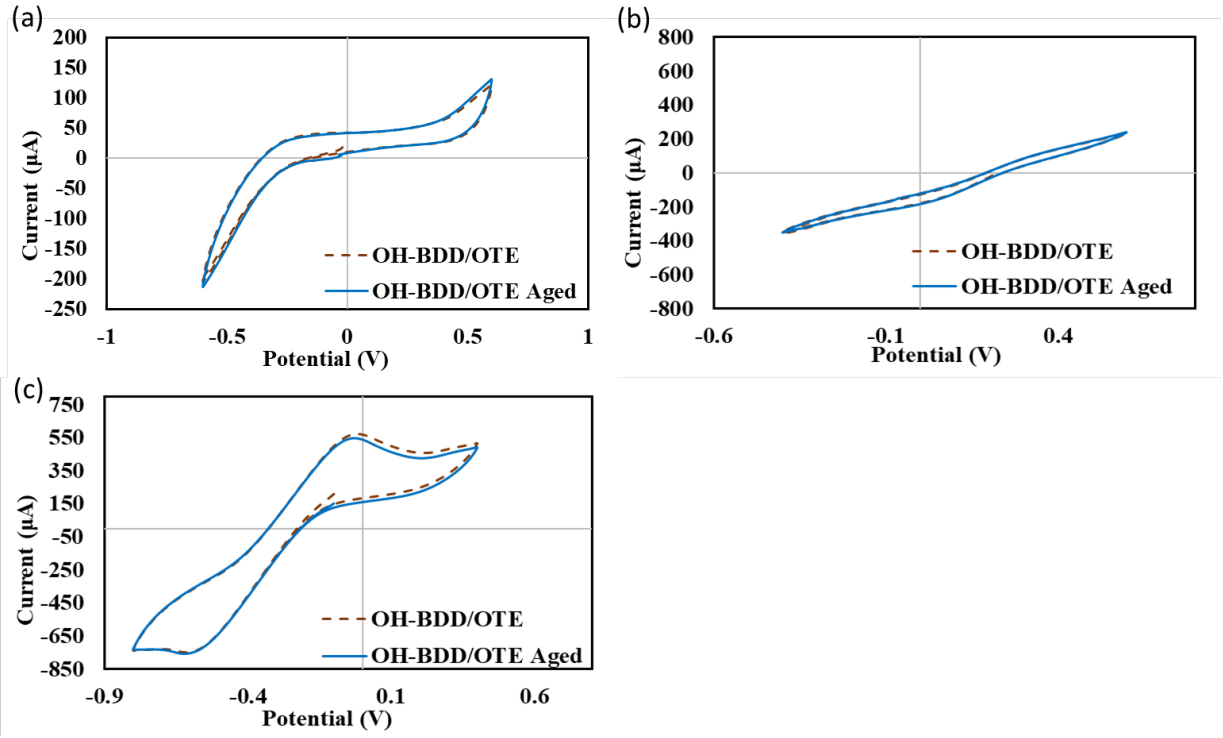

Figure S3. Cyclic voltammogram (CV) curves of bare BDD/OTE and OH-BDD/OTE in the (a) phosphate buffer solution (PBS) (b) PBS containing 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ and (c) PBS containing 5mM $Ru(NH_3)_6Cl_3/Ru(NH_3)_6Cl_2$ before and after aged. (V vs Ag/AgCl)

Titration and data analysis

Titration was applied to determine the pKa values and proton binding site concentrations (N_d) on the bacteria cell surface. The bacteria solution was place in the sealed vessel and purged with N_2 for 30 minutes before titration. The titration solution, 0.1 N NaOH was also degassed for 30 minutes before using. Approximately 4×10^{12} bacteria cells/mL was exposed in the 20 mL 0.1 M NaCl, which was adjusted to pH 2.0 by 0.1 N HCl. The titrations were conducted in the pH range of 2.0 to 11.0 by using an auto-titrator, and a minimum of three titrations were carried out for each experiment.

The pKa values and their corresponding proton binding sites on the bacterial cell surface were determined by fitting the pK_{a_n} and ST_n values into the charge balance equation (S1) using solver in Excel (**Figure S3**).

$$V_{NaOH} = \frac{V_0}{C}\left\{\sum_1^n ST_n \times 10^{-pKa_n}\left[\left(\frac{1}{[H^+]+10^{-pKa_n}}\right)-\left(\frac{1}{[H_0^+]+10^{-pKa_n}}\right)\right]-\left[[H^+]-\frac{K_W}{[H^+]}-[H_0^+]+\frac{K_W}{[H_0^+]}\right]\right\} \quad \text{(S1)}$$

where:

V_{NaOH} = calculated volume of 0.1 N NaOH added in mL

K_w = water dissociation constant (1×10^{-14});

$V_0 =$ Initial solution volume before titration (L); $C =$ concentation of NaOH (N);

$ST_n = Fitting\ parameter\ of\ POA1\ site\ concentration\ (M)$;

$[H^+] = 10^{-PH}; [H_0^+] = initial\ 10^{-pH_0}; n = 4; pKa_n = dissociation\ constant$;

N_{a_i} = site numbers per unit area (#/nm^2), which was calculated according to ($\frac{ST_n}{Total\ cell\ number} \times Avogadro's\ number \times \frac{1}{cell\ surface\ area}$). The total cell number was determined by OD_{600} calibration. The surface area per cell obtained from the measured cell size and the ratio of cell numbers to dry mass were 6.77 μm^2/cell and 7.02×10^{-12} g/cell for POA1, respectively.

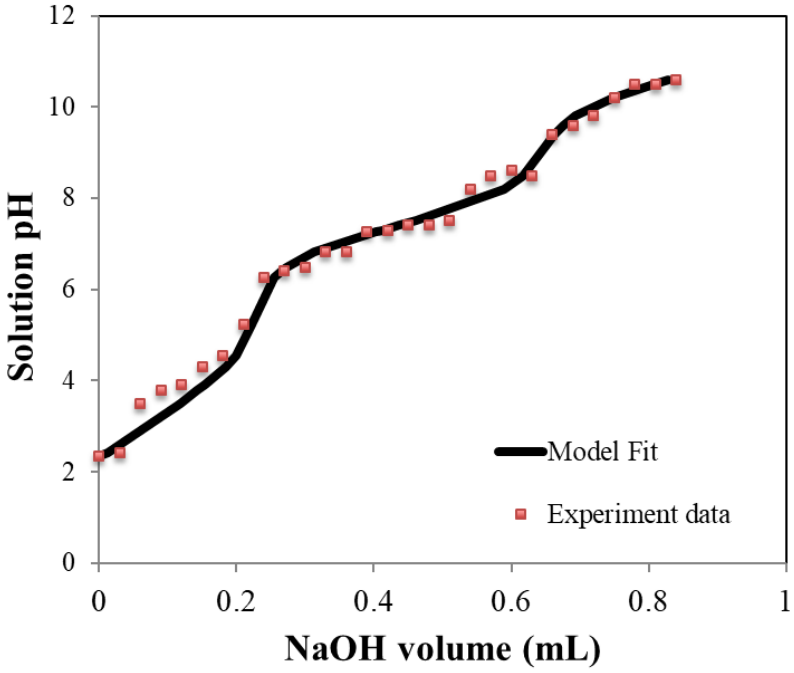

Figure S4. The relationship between solution pH and addition of 0.1 N NaOH volume for POA1 with experiment data and model fitting results.

References

1 C. Park, C. L.-N. Vo, T. Kang, E. Oh and B.-J. Lee, New method and characterization of self-assembled gelatin–oleic nanoparticles using a desolvation method via carbodiimide/N-hydroxysuccinimide (EDC/NHS) reaction, *Eur. J. Pharm. Biopharm.*, 2015, **89**, 365–373.

2 M. K. Walsh, X. Wang and B. C. Weimer, Optimizing the immobilization of single-stranded DNA onto glass beads, *J. Biochem. Biophys. Methods*, 2001, **47**, 221–231.