Supporting Information

Electrochemical biosensor for Staphylococcus aureus detection based on a multilevel surface 3D microstructure

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1. **The relationship between the density of Au clusters distributed on the electrode and the time for electrodeposition**
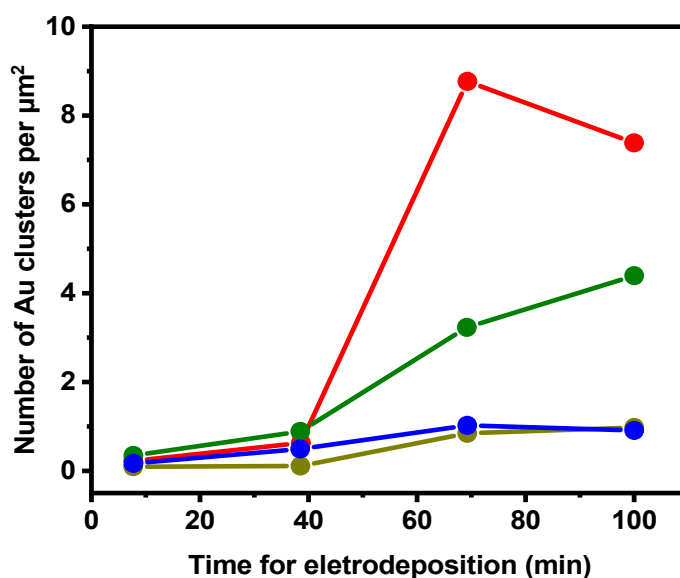

Figure S1 The relationship between the density of Au clusters distributed on the electrode and the time for electrodeposition calculated by their SEM images. Size of the protrusion: 6 μm × 6 μm square (red), 8 μm × 8 μm square (green), 10 μm × 10 μm square (blue), and 16 μm × 16 μm square (brown).
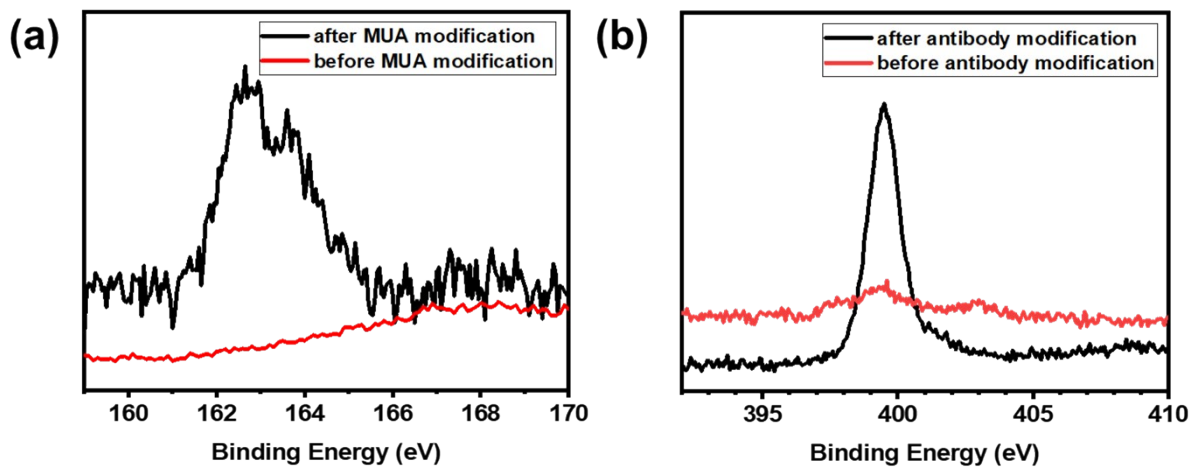
2. Height measurement of the 3D protrusions by AFM

Figure S2 (a) The XPS spectra of S before or after MUA modification. (b) The XPS spectra of N before or after *S. aureus* antibody modification.

3. The EIS spectra of the electrodes that captured *S. aureus* for different lengths of time.
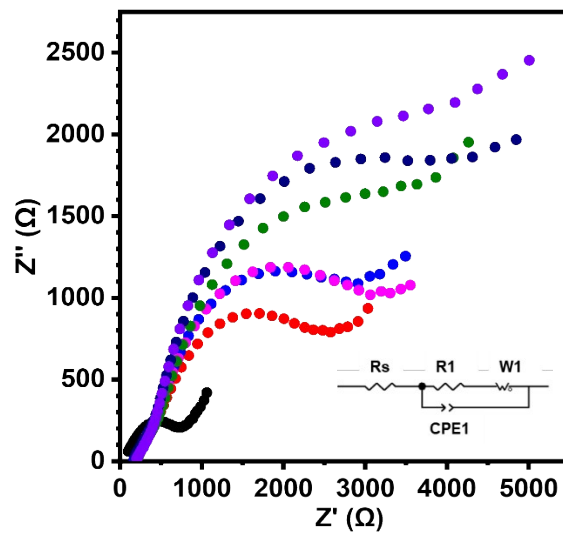

Figure S3. The EIS spectra of the electrodes captured *S. aureus* for different lengths of time. Black plot: 0 min, red plot: 20 min, blue plot: 25 min, magenta: 30 min, green: 35 min, navy: 40 min, purple: 60 min.

4. The EIS spectra of the electrodes which are modified by *S. aureus* antibody solutions with different concentrations.
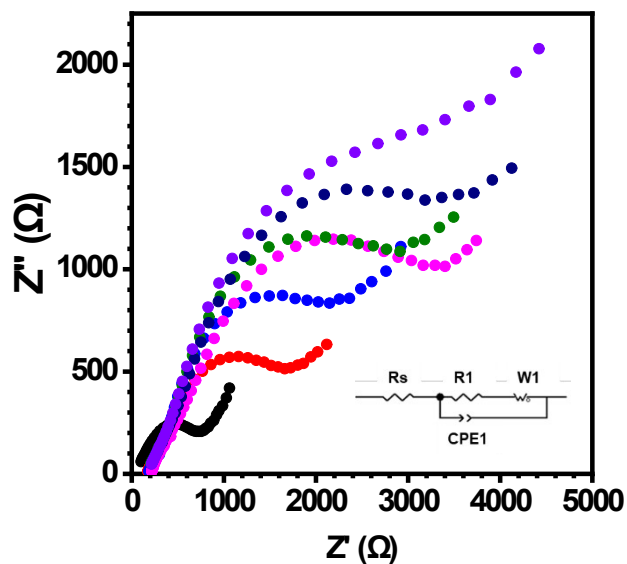

Figure S4 The EIS spectra of the electrodes which are modified by *S. aureus* antibody solutions with different concentrations. Black plot: 0 μg/mL, red plot: 15 μg/mL, blue plot: 20 μg/mL, magenta: 25 μg/mL, green: 30 μg/mL, navy: 35 μg/mL, purple: 40 μg/mL.

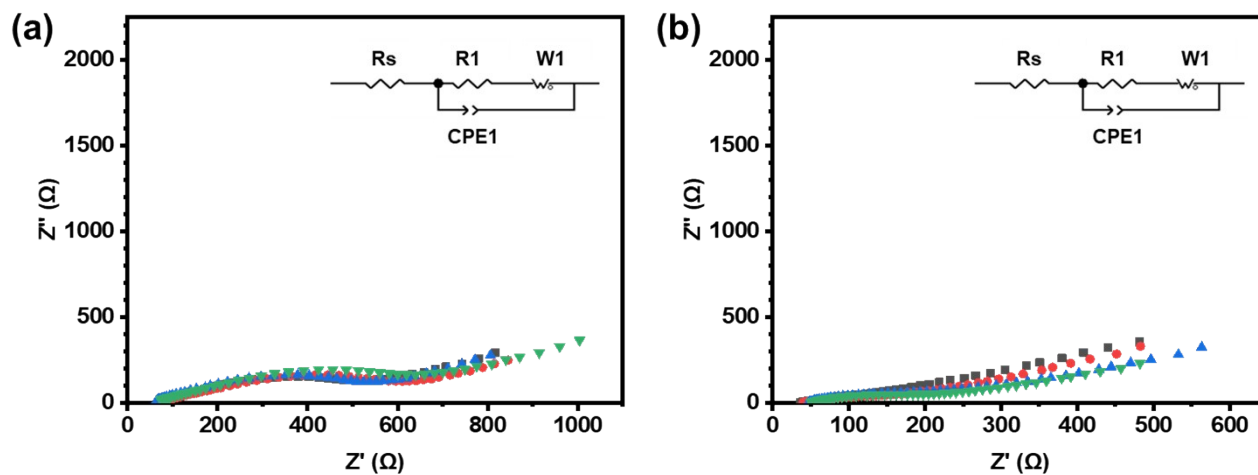## 5. The Change of EIS spectra after absorbing *Salmonella, and E. coli.*

Figure S5. (a) The EIS spectra of the electrodes captured *Salmonella* for different lengths of time. Black plot: 0 min, red plot: 30 min, blue plot: 40 min, green: 60 min. (b) The EIS spectra of the electrodes captured *E. coli* for different lengths of time. Black plot: 0 min, red plot: 30 min, blue plot: 40 min, green: 60 min.

6. EIS spectra of the electrodes that are applied for detecting *S. aureus* in milk and simulated human tissue fluid
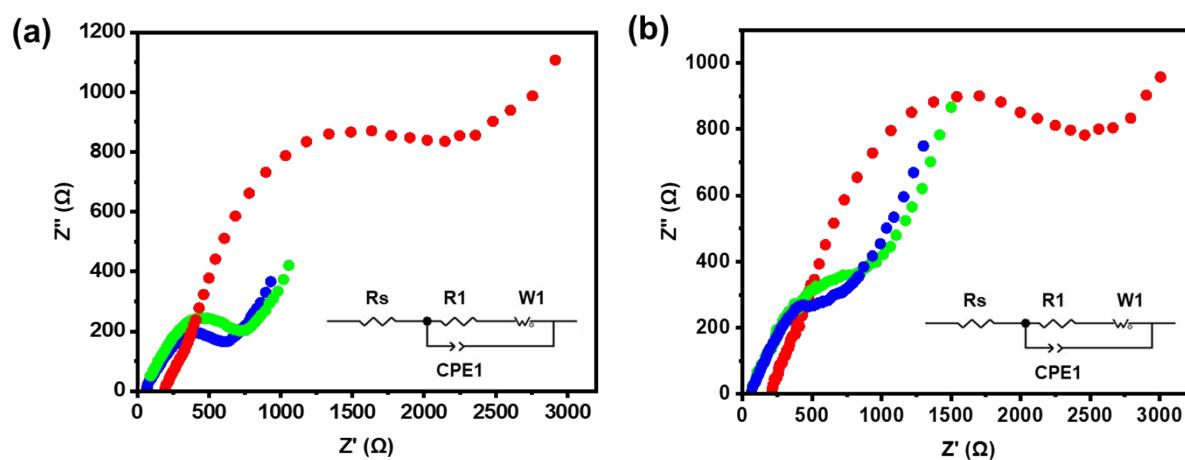

Figure S6. (a) The EIS spectra of the electrodes that adsorbed *S. aureus* from milk containing 0 CFU/mL, 10 CFU/mL, and 100 CFU/mL *S. aureus*. (b) The EIS spectra of the electrodes that adsorbed *S. aureus* from simulated human tissue fluid containing 0 CFU/mL, 10 CFU/mL, and 100 CFU/mL *S. aureus*.