Supplementary Materials

for

Quantitative real-time detection of carcinoembryonic antigen (CEA) from pancreatic cyst fluid using 3-D surface molecular imprinting

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Part 1. Template washing method comparison

For molecular imprinting process, template washing is the most crucial step for the fabrication of molecular imprinting biosensor because it directly determines the number of cavities that can be used for the recognition process. Therefore, the washing method for molecularly imprinted chips has to be optimized. Here we imprinted CEA sensor with the same imprinting condition, and soaked it for 1 hour in three different media: de-ionized water, 1M NaCl and 0.25 M NaOH. To compare the washing effects between 3 treatments, CEA analytes were added into testing solution respectively, and the OCP response vs CEA concentration was plotted in Figure S1 (A). NaCl soaked biosensor generated the largest OCP response up to 60 mV, when DI-water soaked biosensor only generated half of that at around 30 mV. For the one treated with 0.25 M NaOH, biosensor barely generated OCP response with the addition of analytes. The maximum OCP generated during the detection is related to the amount of cavities evolved after the wash following imprinting. Therefore, CV and EIS were employed to characterize the cavity formation. The results are plotted in Figure S1 (B)(C), with the washing effects compared. Figure S1 (B) shows the CV of imprinted CEA sample washed with different solutions. For the sample soaked with DI, no obvious redox peak was generated compared to the untreated CEA imprinted sample. However, when the sample was soaked with 1M NaCl, apparent redox peaks can be achieved, indicating the increase of exposed gold area, which facilitates electron transfer. With the sample treated with 0.25 M NaOH, the redox peaks are almost the same as those of bare gold surface, suggesting that the SAM on gold surface is damaged and washed off by NaOH solution. EIS data shows similar results, which confirm the best washing effect can only be obtained through proper washing procedures. Neither too strong nor too gentle treatment can produce the best performance of MI biosensor.

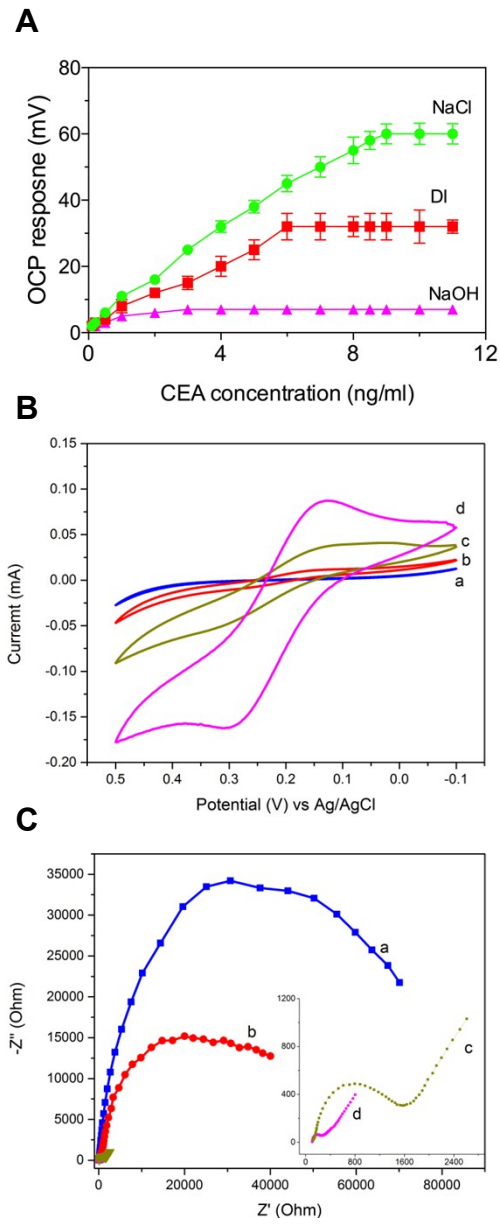
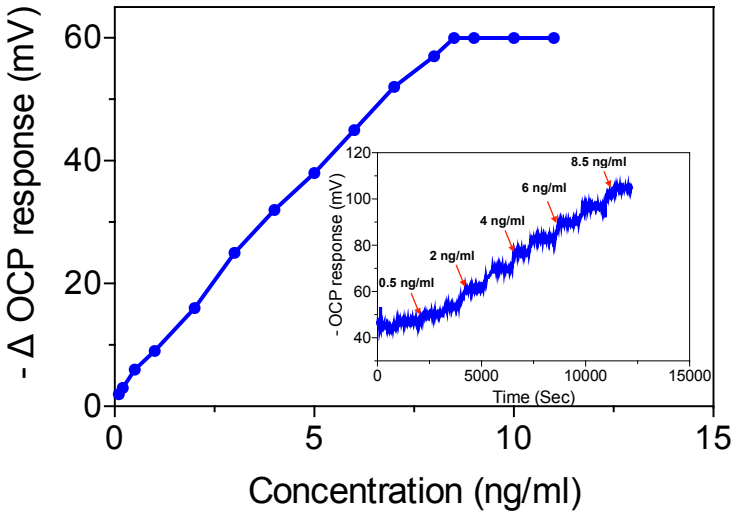


Figure S1 (A) OCP response of CEA sensor fabricated using 3 different washing media: NaCl, DI water and NaOH. (B) Cyclic voltammograms (CV) of the different electrodes: (a) after imprinting with CEA, imprinted CEA sensors soak with different media: (b) NaOH, (c) NaCl, (d) DI water. Scan rate, 0.1 V/s. (C) Electrochemical impedance spectra (EIS) of different electrodes: (a) After imprinting with CEA, imprinted CEA sensors were soaked with different media, (b) NaOH, Inset: (c) NaCl, (d) DI water. EIS was tested with 5 mV amplitude and frequency range of 0.1-100,000 Hz. Both CV and EIS were performed in KCl solution (0.1M) containing $Fe(CN)_6^{3-/4-}$ (5 mM).

Part 2. CEA real time OCP detection

Potentiometer was employed to monitor the OCP response for the analyte detection. In Fig. S2, the OCP response increases with the addition of CEA detection solution. As the surface cavities were fully covered by CEA analytes, the OCP response reaches the maximum value. CEA concentration in detection beaker vs - Δ OCP response was converted from the original Time vs – OCP response data. In the inset of Fig. S2, the OCP response process was monitored by potentiometer. CEA detection solution was added into detection beaker stepwise by micropipette, and the OCP response was recorded by potentiometer. The



concentration of each addition was labeled in the figure. Through the data monitored by potentiometer, the CEA detection process can be easily recorded and further analyzed.

Figure S2. CEA real time OCP detection. OCP response as a function of CEA concentration added into testing beaker. Inset was the original Time-OCP response data. The OCP response was plotted as a function of time period. The dynamic process of OCP detection can be monitored through potentiometer.