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Investigation of cleaning and regeneration methods for reliable construction of DNA cantilever biosensors

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Abstract: Biosensing systems based on detecting changes in cantilever surface stress have attracted great interest. To achieve high reliability of measurements, high quality and high reproducibility in functionalization of the sensor surface are key points. In this paper, we investigate different methods to clean and regenerate the sensing surface of cantilever biosensors. Perchloric acid potential sweep, potassium hydroxide-hydrogen peroxide, and piranha cleaning are investigated here. Peak-current potential differences from cyclic voltammetry, X-ray photo-electron spectroscopy and fluorescence detection are applied to characterize surface cleanliness. The experimental results show that piranha cleaning is the most reliable and efficient cleaning procedure.

Keywords: Surface Cleaning, DNA Biosensor, Cyclic Voltammetry

1. INTRODUCTION

Immobilization of single-stranded DNA (ssDNA) on surfaces is the basis for many biosensing applications. In most cases, DNA is immobilized on, e.g. gold, glass, silicon nitride, or oxidized silicon [1]. An important requirement for the functionalization of biosensors is to obtain a clean and stable sensing surface. Furthermore, it would be necessary to regenerate the surface for recycling the chip. Many techniques have been developed for cleaning gold surfaces [2]. Cantilever biosensors are often coated on one side with an nm thin gold film and an adhesion layer. Some of the well-known cleaning techniques may not be suitable for cleaning fragile cantilever structures with thin metal coatings. In this work, we investigate different cleaning methods to ensure the cleanliness of the thin gold surface and regenerate the surface after ssDNA modification. The best cleaning method is identified by the results of cyclic voltammetry (CV), X-ray photo-electron spectroscopy (XPS) and fluorescence labelling.

2. EXPERIMENTAL

The chips with gold top electrodes (using chromium as adhesion layer) were fabricated on a silicon nitride coated wafer using standard cleanroom processing. A CH Instruments electrochemical analyzer was used to perform CV. Before and after treatment with different cleaning methods, each chip was characterized with CV by sweeping the potential from 600 mV to -200 mV with 20 mV/s rate in 1mM $[Fe(CN)_6]^{3-/4-}$ redox couple with 200 mM KNO_3 solution. Elemental information of the electrode surface before and after DNA functionalization was acquired using a ThermoFisher Scientific K-Alpha X-Ray Photoelectron Spectrometer. Fluorescence detection of the labelled ssDNA was used to evaluate the efficiency of surface regeneration.

Thiol-modified 25-mer ssDNA and poly(T)poly(C)-tagged DNA oligonucleotide probes were applied to modify the gold surfaces.

Potential sweep in perchloric acid : The chips were placed in a three-electrode electrochemical cell and the potential was cycled between 0 mV and 1600 mV at 20 mV/s scan rate in 0.1 M $HClO_4$ until a stable CV was obtained. Potassium hydroxide/hydrogen peroxide cleaning: The chips were placed in a solution of 50mM KOH and 25% H_2O_2 for 8 min. Piranha cleaning: The chips were kept in piranha solution (30% H_2O_2 and concentrated H_2SO_4 in the ratio of 1:3) for 8 min.

3. RESULTS AND DISCUSSION

The difference between the potential of the cathodic and anodic peak of a CV, ΔEp, is a useful diagnostic test of electrode quality. For the redox process of a reversible redox couple, such as $[Fe(CN)_6]^{3-/4-}$, on perfectly clean electrodes at steady state, ΔEp = 58 mV at 25 ^oC [3]. As shown in Figure 1, the samples exhibit an average value of ΔEp = 117 mV before cleaning, and after cleaning in $HClO_4$, KOH+H_2O_2 and Piranha, the ΔEp values are140 mV, 110 mV and 79 mV, respectively. The results indicate that in this study, Piranha cleaning is the most effective one among all the methods for generating clean gold surfaces.

Figure 2 shows microscopy images of the chip surfaces after modification with and removal of the fluorescently labelled poly(T)poly(C)-tagged DNA. Clearly, Piranha cleans very well both the gold and silicon nitride surface. It is suggested that Piranha can remove the DNA samples completely to facilitate a reproducible recycling of the sensing surfaces. In Figure 3, XPS results obtained on the chips before and after immobilization of thiol-modified ssDNA confirm the fluorescence results for the three methods ($HClO_4$ cleaning not shown in this paper).

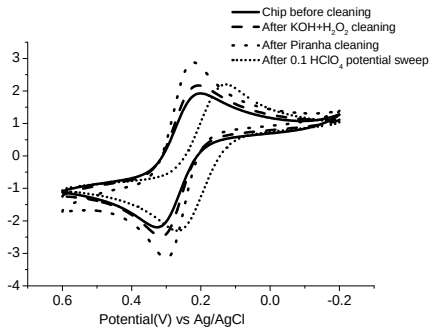

Figure 1 Comparison study of CV scans obtained on the gold electrodes before cleaning, and after cleaning with $KOH+H_2O_2$, Piranha, and potential sweep in $HClO_4$.

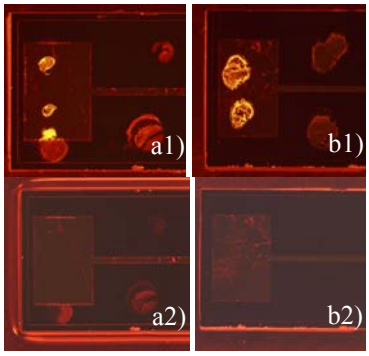

Figure 2 Fluorescence images acquired on: a1) and b1) gold electrodes with surrounding insulating silicon nitride layer after modification with poly(T)poly(C)-tagged DNA oligonucleotide probes; the same chips after cleaning with a2) $KOH + H_2O_2$ and b2) Piranha.

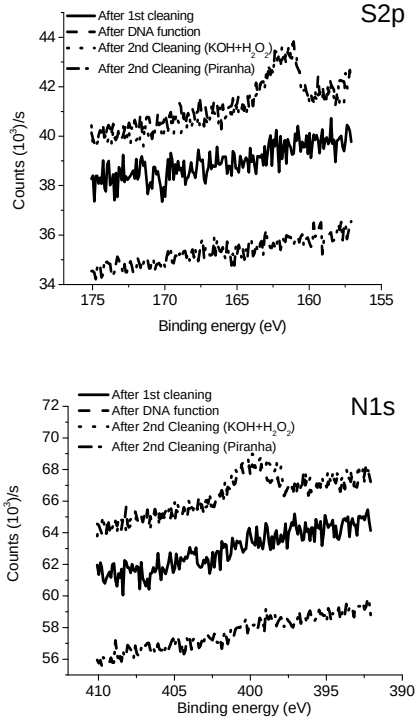

Figure 3 XPS results obtained on the chips after cleaning, DNA modification and re-cleaning.

4. CONCLUSION

The main conclusion of this study is that Piranha cleaning is the most effective method facilitating chip recycling.

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