A NOVEL REUSABLE NANOMECHANICS-BASED PROTEIN BIOSENSOR WITH ELECTRICAL MANIPULATION

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ABSTRACT

A novel reusable biosensor of label-free biomolecular recognition based on the nano-mechanics transduction in a micro-fabricated cantilever has been developed. The nanomechanics-based immunoassay biosensor was proven to be repeatedly used with desorption of the pre-bound antigen-antibody by an in-situ alternative electrical field. This new approach of electric manipulation in separating the antigen out of the antibody in a buffer fluid environment was confirmed in nanomechanics-based immunoassay biosensor in which a conventional desorption is long used with high concentrated acid solution in a range of pH 2~3. The potential benefit of the electrical means over a conventional pre-bound antigen-antibody desorption of strong acid solution is to maintain long-standing protein activity in multiple uses such that the micro biosensor is reliable and reproducible in every detection. The complete sensing history from a monolayer of biolinker, to antibody and antigen is also first demonstrated in nanomechanics-based immunoassay.

1. INTRODUCTION

It is becoming gradually obvious that the high-throughput recognition and quantification of bio-molecules are of great importances in biomedical detection and disease diagnosis. With the growing interest and fast development in bio-nanotechnology, bio-sensing tools have been moving towards miniaturization, high sensitivity, and great promise in low-cost as well. Recent papers have reported the observation that as specific bio-molecular interactions occur on one surface of the micro-cantilever beam, the device is gradually deflected due to bio-induced surface stresses. Several research teams have utilized the device surface stress-induced deflection for detection of the monolayer coverage [1], the DNA hybridization [2] and the antigen-antibody binding [3-4]. As a result, the specificity and interaction of biomolecular recognition are able to be transduced and even monitored in such a micro-cantilever beam device. Moreover, the device offers several advantages such as use of only small quantities of molecules for bioassay, label-free detection, potentially high parallelization, and mass production.

We report on a new reusable biosensor which accomplishes both the bio-specific binding detection of the micro-mechanical cantilever and the electrical control desorption of pre-bound antigen-antibody, as shown in Fig.1. The present study has successfully demonstrated that the bio-induced surface stress nano-mechanics may interpret the bio-molecular recognition between antigen and antibody. Moreover, an alternative electric field was first employed to depart the anti-biotin from a biotinylated SnO_2 electrode rather than using chemically the high concentrated acid for the bio-detection regeneration, which showed relatively long-standing protein activity [5]. With the merit of MEMS technique that allows highly compatible integration of nano-mechanical detection with electric devices, the electrical manipulation is first embedded into the nanomechanics-based antigen-antibody bio-detection for physical desorption, thus resulting in potentially reusable

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immunoassay diagnosis, miniaturization, high sensitivity, and high parallelism.

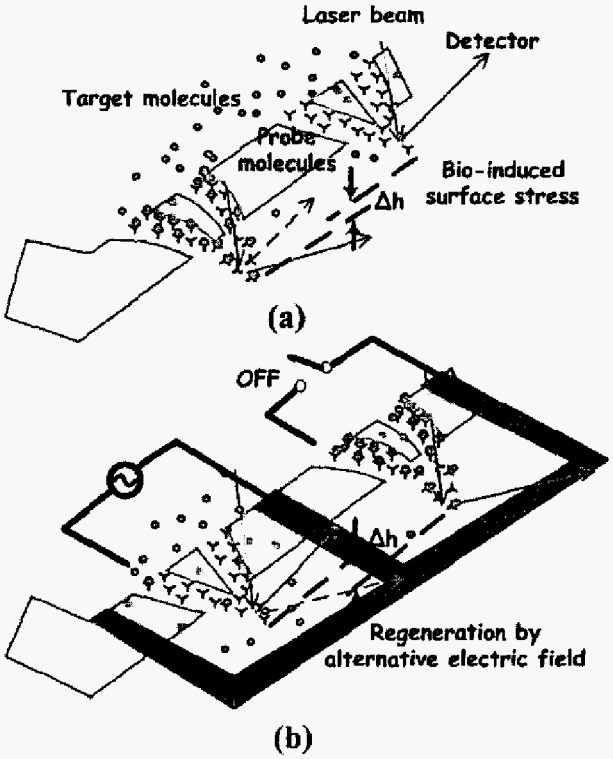

Figure 1: (a) Specific bio-molecular interactions between target and probe molecules result in bending of a micro-cantilever. (b) Departure of target molecules from probe molecules by an alternative electric polarization field.

2. MATERIALS AND METHODS

Micro-cantilever bio-detection chip fabrication

The present work consists of both a protein-chip device inside a micro-fluidic channel and protein interaction detection system. First, the protein sensor device was made based on the translation of bio-molecular recognition and interaction onto nanomechanics of bio-induced surface stresses. The micro-cantilever device made with a V-shaped silicon nitride layer (length = 200 μm, width = 40 μm, and thickness = 0.6 μm) and an evaporated 25-nm gold top layer was micro-machined on a silicon substrate. Meanwhile, the V-shape silicon nitride layer serves as a mechanical structure, and the top gold layer is to react chemically with a bio-linker of self-assembled mono-layers (SAMs). The protein biochip inside the micro-channel was completed as

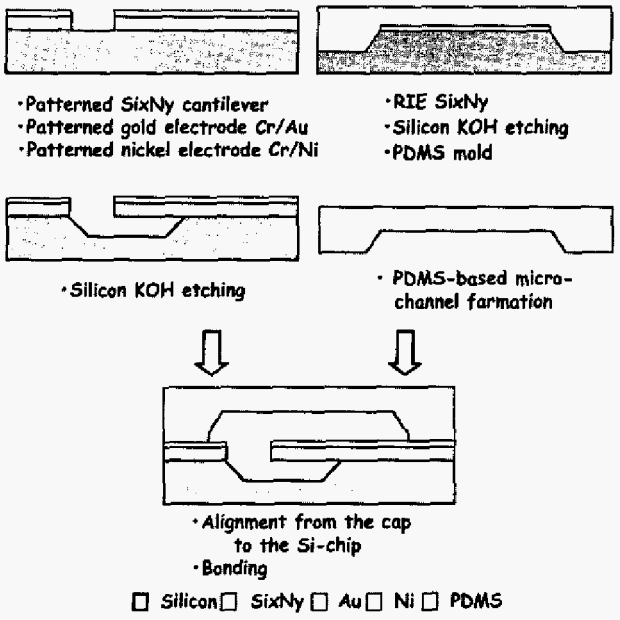

Figure 2: Fabrication process flow of micro-cantilever bio-detection chips.

the diced chip was bonded with a recess-preformed PDMS-based channel structure that is also transparent to the optical detection system built outside of the device.

The detailed fabrication process flow is shown in Fig. 2. In addition, the electrode of electrical manipulation for pre-bound antibody-antigen desorption was made with an evaporated 25-nm nickel layer which would not chemically react with the SAMs. Fig. 3(a) shows the SEM picture of the micro-cantilever and its associated electrodes in device-level arrangement. The full view of the micro-fluidic system integrated with micro-cantilever bio-sensing device inside is shown in Fig. 2(b). Meanwhile, the complete micro-fluidic system was attached to an upstream well-controlled flow-rate syringe pump and a downstream reservoir.

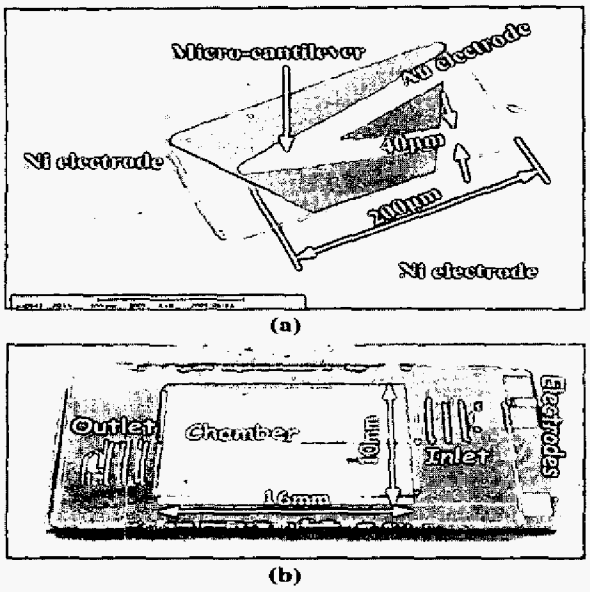

Figure3: (a) The SEM micrograph shows the micro-cantilever and its associated electrodes. (b) A complete micro-cantilever bio-detection chip.

Experimental setup and approach

The detection system for antigen-antibody recognition-induced deflection of the micro-cantilever utilizes an optical displacement-sensitive detection device for the biochip characterization. In this optical detection system, a laser beam emitted from a He-Ni laser was focused onto the tip of the V-shape micro-cantilever, and then reflected to a position-sensitive detector (PSD). Due to the interaction of the antigen and antibody, the resulting displacement of the reflected laser beam can be detected by the PSD so that the antigen-antibody recognition can be characterized through the biochip and this optical system.

Cantilever surface fuctionalization

Fig. 4 shows the chemical and antigen-antibody process flow of the ImmunoglobulinG1 (IgG1) assay in which the micro-cantilever real-time motion was optically monitored at every bio-chemical reaction step. At first, a self-assembled monolayer of carboxylic acid-terminated alkylthiol was injected into the micro-channel. After the binding was completed, rinsed thoroughly with PBS, and an activator of one mixture was injected, forming a suitable surface for adsorption of anti-IgG1. Then the device was rinsed with PBS again and covalently immobilized the anti-IgG1. After the immobilization was completed, rinsed with PBS again , and the ethanolamine-HCl was injected to be used as an agent to inactivate the nonspecific binding sites of a functionalized surface.

Antigen and antibody interaction

After finishing the surface functionalization of the micro-cantilever, the IgG1 of antigen was injected and thus interacted with the antibody. The next experiment of desorption used to flow high concentrated acid to depart antigen out of the immobilized antibody. Up to this process, a complete process is done completely and ready for next antigen-antibody interaction procedure. As described earlier, the physically electrical manipulation of desorption is able to maintain long-standing protein activity. In this reusable sensor, the alternative electric polarization fields with different voltages to bound IgG1 and anti-IgG1 was conducted to investigate its desorption of IgG1 out of the anti-IgG1.

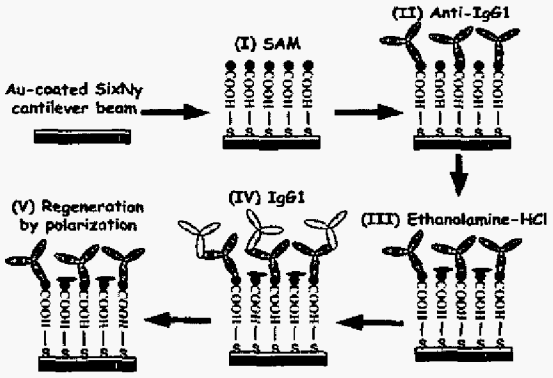

Figure 4: Illustration of IgG1 assay.

3. RESULTS AND DISCUSSION

As a result, a complete historic spectrum of nanomechanics-based signal to the bio-molecular recognition and its bio-molecular regeneration process of electrical manipulation were successfully obtained, as shown in Fig. 5. Change in beam defection and surface

stress with respect to time can be obtained from Stoney's formula [6]. Meanwhile, the bio-molecular recognition of anti-IgG1 to IgG1 was found that a time-elapsed micro-cantilever bend-down deflection, and the long time-scale biofilm-induced tensile stress results from a gradual rearrangement of the adsorbed protein. By giving alternative electric fields with different voltages, the nano-mechanical surface stress was released back to its unbound state. As indicated in Fig. 6, giving electric polarization fields with higher voltage resulted in fast response of the antigen-antibody desorption in which providing a ±10V of the 0.2Hz square wave of alternative electric fields lead to a remarkable response comparing to that in ±1V.

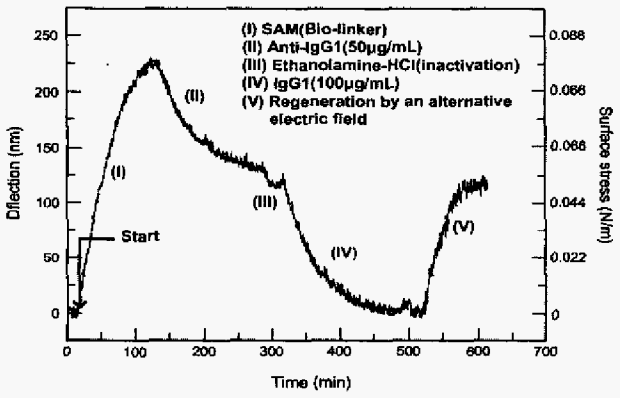

Figure 5: The historic deflection of the micro-cantilever in response to the biomolecular binding reaction.

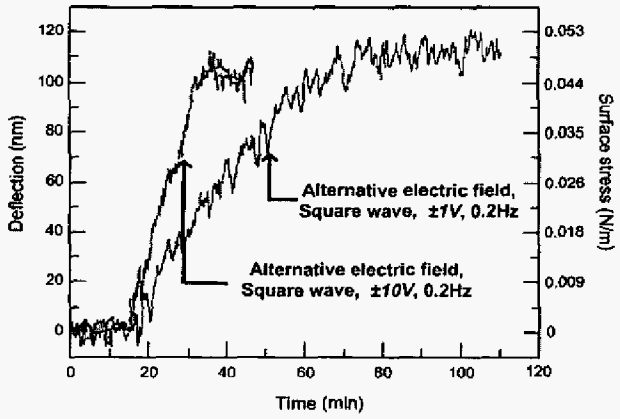

Figure 6: Comparison of the cantilever deflection as a function of time for giving different electric polarization fields.

4. CONCLUSION

In summary, based on the results obtained in this work, the nanomechanics-based biosensor exhibits a complete historic spectrum of nanomechanics-based signal to the bio-molecular recognition and its bio-molecular regeneration process. The electric manipulation of antigen desorption out of the immobilized antibody in nano-mechanics-based biosensors was proven in buffer fluid environments. Finally, the nanomechanics-based biochip has demonstrated its high applicability, effectiveness, miniaturization, and potentially high parallelism.

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