MULTI-TARGET TOXIN DETECTIONS BASED ON PIEZORESISTIVE MICROCANTILEVERS
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ABSTRACT
In this paper, we designed and fabricated piezoresistive cantilevers with a signal-noise-rate (SNR) up to $10^4$ by optimizing the structural dimensions and the process parameters. By using the cantilever-based sensors, staphylococcal enterotoxin B (SEB) with the concentration of 1 nM was measured by using thiol-bonding-aptamer functionalized cantilevers, and ricin with the concentrations of 0.14 nM and 0.28 nM were also detected by utilizing biotin labeled polyclonal antibody (PcAb) functionalized cantilevers. Measurement results shown that the cantilever-based sensors have sufficient sensitivity and stability to detect toxin as low as a few pMs and may provide a platform for detection of protein-protein binding, oligo-protein interaction and DNA hybridization.

KEYWORDS: Toxic, Piezoresistive cantilevers, SEB, Ricin, Aptamer

INTRODUCTION
It is becoming increasingly obvious that rapid and quantitative method for detection of toxins is needed for public health protection. Many researches have indicated that intermolecular nano-mechanics bend microcantilevers when binding occurs on the surface of cantilevers between specific biomolecules, which can be optically/piezoresistively detected. Compared with optical readout system, piezoresistive cantilevers working in a static mode reveal advantages of low cost, portability and easy implementation for sensing in liquid environment. Aptamers, artificial oligonucleotides that can bond to a wide range of target molecules, exhibit many advantages such as low molecular weight, amenability to modifications and chemical stability, which leads to apta-biosensors with high sensitivity and specificity. Furthermore, surfaces functionalized with streptavidin-biotin system indicates affinity 3-8 times greater than traditional antibody-antigen method. In this paper, piezoresistive cantilevers with high SNR were fabricated, and SEB as well as ricin was detected by using thiol-bonding-aptamer and streptavidin-biotin functionalized cantilevers respectively.

CANTILEVERS DESIGN AND FABRICATION
Piezoresistive microcantilever is operated for the detection of surface stress change by measuring the static deflection of microcantilevers. Fig. 1 describes the formation of surface stress on microcantilever surface when bio/chemical molecules absorbed on the immobilized layer. In piezoresistive cantilever biosensor, the change of resistance can be measured using a Wheatstone bridge. The relative resistance change ($\Delta R/R$) of a piezoresistor at the Wheatstone bridge is described by the equation.

$$\frac{\Delta R}{R} = \beta \frac{3\pi_t(1-\gamma)}{t} (\sigma_1 - \sigma_2)$$

Where $\pi_t$ is the piezoresistive coefficient, $\sigma_1$ and $\sigma_2$ are the longitudinal stress and transverse stress respectively, $t$ is the thickness of cantilever, $\gamma$ is the Poisson’s ratio, $\beta$ is a coefficient that adjusts for the thickness of the piezoresistor.

In our design, four piezoresistors with two of them located on measurement cantilevers and other two on the reference cantilevers compose a half Wheatstone bridge sensor. Rectangular cantilever with the dimension of 200 $\mu$m $\times$ 50 $\mu$m $\times$ 1 $\mu$m and the piezoresistor’s leg dimension of 100 $\mu$m $\times$ 13 $\mu$m was designed. 50 nm thick gold film was coated on the surface of measurement cantilevers for the surfaces functionalization.
The process used to fabricate the piezocantilevers are shown in Fig. 2. SOI wafers with the layer thickness of 0.34/0.4/525 μm, p-type, in [100] orientation were used. In order to increase the SNR of the sensor, some improvements have been made during the fabrication. Firstly, an N+ ring was designed around the P- piezoresistor to avoid the electrical leakage of the piezoresistor. Then, a doping dose for the piezoresistor with a 7 kΩ resistance was optimized and P+ doped silicon was used as electrical connection instead of metal, which could decrease the thermal drift of the sensor. The third, a 30 nm SiO₂ layer was deposited as the protective layer during the ion implantation to reduce the surface damage brought by the ion bombardment. Finally, a hybrid process of anisotropic and isotropic dry etching was adopted to release the cantilevers, which can increase the reliability and avoid the contamination of potassium ions that may affect the stability of the output signal. With these improvements and the optimizations on process parameters, piezoresistive cantilever sensors with the SNR up to 10⁴ have been obtained. The scanning electron microscope (SEM) image of the fabricated device is revealed in Fig. 3.

EXPERIMENTAL

In our experiments, all solutions were prepared with double-distilled water. N-hydroxy-succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. 3,3’-dithiopropionic acid (DDPA), phosphate buffered saline (PBS), streptavidin, ethanolamine were obtained from Solarbio Ltd. (Beijing, China).

Prior to probe immobilization, the cantilevers were first pretreated with oxygen plasma (250 W, 52 sccm) for 1 min, and then entirely cleaned with piranha solution (3:1 sulfuric acid to hydrogen peroxide) to remove any organic contamination. For anti-SEB immobilization, the cantilevers were immersed in 20 μg/mL anti-SEB solution at 4 °C for 24 h allowing the formation of a self-assembled monolayer (SAM) through covalent bond of the thiol-group with the gold film, as shown as in Fig. 4(a).

In the functionalization process of ricin PcAb, streptavidin-biotin sensor labeled modification was performed at room temperature, as shown in Fig. 4(b). First, a SAM of DDPA (5 mg/mL) was formed on the gold surface for 30 min. Then the cantilevers were immersed in a solution with 10 μg NHS and 10 μg EDC for 20 min. After the streptavidin (100 μg/mL, 100 μL) was cross-linked to the semi-stable amine-reactive NHS ester, remaining carboxyls (1 M, 100 μL) were inactivated with ethanolamine. The functionalization process was completed by adding biotin labeled ricin PcAb probe (3.071 mg/mL, 30 μL) into 300 μL PBS (pH7.4, 0.01 M) over 1 h.

When the probes immobilization of SEB and ricin were performed on the gold surface, the cantilevers were rinsed three times with PBS to remove the excess probes. After the PBS washing, the cantilevers were connect to the measuring ...
instrument to obtain a stable base line of the output signal. Finally, 15 μL SEB solution with the concentration of 1 μg/mL was added to 480 μL PBS for antigen-bonding detection at room temperature, which corresponds a concentration of 1 nM. For the detection of ricin, 30 μL ricin solutions with the concentrations of 100 ng/mL and 200 ng/mL were added to a 300 μL PBS separately, which correspond the detection concentrations of 0.14 nM and 0.28 nM respectively.

RESULTS AND DISCUSSIONS
In order to demonstrate the performance of cantilevers, SEB and ricin detections were realized separately. The immobilization processes of antigen-bonding were monitored in real time individually. The curve in Fig. 5(a), which is the signal output of the microcantilever sensors, shows the bonding process of SEB and anti-SEB. As in the experimental protocol described above, the responding concentration of SEB was 1nM. The saturation time was about 500 seconds with a 20μV output change.

For the ricin detection, the signal output changes of 670 μV and 1500 μV were obtained separately which correspond the detection concentrations of 0.14 nM and 0.28 nM. The measurement results for two ricin concentrations are shown in Fig. 5(b) and Fig. 5(c), we can observe that the signal output of cantilever sensors reaches a saturation state for the two curves. It is only a few minutes for the higher concentration ricin, but the saturation time is much longer for the lower concentration ricin. It is obvious that this is determined by the collision theory. When the concentration is much higher, more intermolecular effectual collisions are brought between probes with antibody and hence saturation state needs for a shorter time.

CONCLUSIONS
A piezoresistive cantilever biosensor with high sensitivity was successfully fabricated and the detections of SEB based on thiol-bonding-aptamer functionalization and ricin based on biotin labeled PcAb bonding to streptavidin functionalization were realized. With attempts to optimize the cantilevers processes, it is possible to detect two toxins as low as 1nM and 0.14nM with thiol-aptamer and streptavidin-biotin functionalized cantilevers. Next work we will focus on to immobilize the probes quantificationally and achieving lower limit of detection (LOD) of toxin identification. We believe that the piezoresistive cantilever sensors can be applied for the detection of other protein-protein binding, oligo-protein interaction and DNA hybridization as low as a few pM in further investigation.

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REFERENCES

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