Preparation of a Novel Microcantilever Array Biochemical Sensor

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Abstract: To eliminate the impact of environmental noise such as temperature drift and change of liquid refractive index in the single microcantilever biochemical sensing system and achieve rapid parallel detection of various target molecules, a novel microcantilever array biochemical sensor was designed and constructed. When the microcantilever array is scanned by a piezoelectric-driven laser beam, the kinetic curves of the specific biochemical reaction on the microcantilever array surface is obtained by real-time monitoring the deflections of the microcantilevers with the optical lever read-out technique. In the experiment, the system optical stability was verified by 9 h scan of two fixed points with 250 μm pitch, and also the system detection reliability was verified by the temperature excitation test, in which the microcantilever array deflections were basically consistent with each other (error 6.5%) when heating up 6 °C. Finally, we detected 10 μg L⁻¹ of clenbuterol antigen by immobilizing clenbuterol antibody on the gold-coated side of the microcantilever array with the self-made capillary array modification device, which verified the practical feasibility of the microcantilever array sensor.

Key Words: Biochemical sensor; Label-free; Microcantilever array; Piezoelectric-driven; Optical lever; Capillary array

1 Introduction

Microcantilever biochemical sensors are developed based on the micro-electro-mechanical systems and atomic force microscopy[1,2], and have the advantages of high sensitivity, label-free, in-situ reproduction of biochemical reaction information, etc. In addition, its detection principle is as following: when the biochemical reactions occur on the microcantilever unilateral surface, the surface stress will change, which makes the microcantilever bend. Then we can obtain the information of the biochemical reactions by detecting the microcantilever deflection with optical or electrical methods. In last decade, the application of the microcantilever sensing technology gradually changes from the initial humidity and temperature measurement[3] to the biological engineering[4] and environmental pollution monitoring[5], etc. Since 2002, we completed the detection of conformational changes of Poly (N-isopropyl acrylamide)[6], Cu²⁺[7], clenbuterol, chloramphenicol[8], paclitaxel[9] and others by this sensing technique.

To eliminate the impact of environmental noise such as temperature drift and change of liquid refractive index in the single microcantilever biochemical sensing system[10] and achieve rapid parallel detection of various target molecules, a novel microcantilever array biochemical sensor was designed and constructed. At present, the reported microcantilever array sensing methods mainly include optical interferometry detection method[11], timing scan detection method of vertical cavity surface emitting lasers (VCSELs)[12], CCD detection method of expanded light source[13], etc. These methods have their advantages and disadvantages. The optical interferometry detection method has high sensitivity, however, its harsh
testing conditions and requirement of high anti-vibration performance make it difficult to be applied in practice; VCSELs timing scan detection method can sensitively detect eight microcantilevers, however, its unadjustable emission intervals make it inflexible, also the cost is expensive. CCD detection method of expanded light source can do multi-channel detections, however, the detection sensitivity is not high because the image dispersion induced by microcantilever bending seriously affects the detection quality of the spot displacement.

In this study, we presented a microcantilever array biochemical sensing method based on piezoelectric scan principle. In comparison with the existing detection methods, this method has the advantages of simple principle, high sensitivity, easy to adjust and fast positioning microcantilever array with any interval. Meanwhile, we designed a capillary array modification device that could effectively modify the biochemical molecules on the microcantilever array. In addition, the modification device has the advantages of higher modification efficiency and lower cost than that of the commercial point-sampler instrument. Finally, the sensor successfully eliminated the impact of environmental noise such as temperature drift and change of liquid refractive index.

2 Detection principle

By the microcantilever biochemical sensing method, we realized the monitoring of the biochemical reactions occurred on the microcantilever unilateral surface by detecting the change of the microcantilever surface stress. According to the Stoney formula\textsuperscript{[14]}, the relationship between the microcantilever tip displacement $\delta$ and its surface stress change $\Delta\sigma$ can be expressed as following:

$$\delta = \frac{3(1-\nu)l^3}{Et^2} \Delta\sigma$$ (1)

where, $l$, $t$, $E$ and $\nu$ is the length, thickness, Young’s modulus and Poisson’s ratio of the microcantilever, respectively. As shown in Eq.(1), when the material and geometric parameters of the microcantilever are confirmed, $\delta$ will be directly proportional to $\Delta\sigma$, and then the change of the microcantilever surface stress can be observed by detecting its tip displacement, so that the corresponding biochemical reaction information can be detected.

At present, the methods for detecting the microcantilever deformation mainly include capacitance, piezoresistive, optical and electromagnetic detection methods\textsuperscript{[11]}. Among them, the optical lever method has been most widely used because of its advantages of simple structure, high sensitivity and the order of $10^{-11}$ m of vertical resolution\textsuperscript{[15]}. Figure 1 shows the principle of accurate detection of biochemical reactions on the microcantilever array. In biochemical reactions, we used the reference microcantilever modified inert molecules (not involved in biochemical reaction) to detect the environmental noise signal. After reaction, the microcantilever deformation signal generated only by the specific binding of probe molecules and target molecules can be obtained from the differential deformation signal of the detection microcantilever and the reference microcantilever.

3 Results and discussion

3.1 System design

According to the piezoelectric scan principle, we designed a microcantilever array biochemical sensing system (shown in Fig.2a). The piezoelectric ceramic tube drives the fixed convex lens to do periodic reciprocating motion (Fig.2b).

![Fig.1 Schematic of micro-cantilever array detection](image)

![Fig.2 Schematic of micro-cantilever array biochemical sensor (a) and schematic of piezo-driven scanning (b)](image)
While the laser beam passing through the convex lens scans the microcantilever array, we obtained the deformation signals of the microcantilever array by using the PSD to receive the reflected light position signals in sequence, thus monitored the biochemical reactions on the microcantilever surface in real-time.

Figure 3 shows the schematic diagram of the convex lens deflecting to drive the laser beam to scan the microcantilever array. When the piezoelectric ceramic tube deflected with its input voltage change, the convex lens fixed in it will deflect, too. If the center position of the convex lens moves from O to O', the converged laser focus will accordingly move from F1 to F2. Then the interval and frequency of the scan laser beam can be accurately controlled by means of setting the magnitude and the period of the input voltage on the piezoelectric ceramic tube.

3.2 Test of scan light path stability

First, we fixed a commercial microcantilever array (Micromotive, Germany, shown in Fig.4) into the detection system. The dimensions of the microcantilever array were 500 µm in length, 90 µm in width, 1 µm in thickness and 250 µm in pitch, and one side of these cantilevers were covered with a thin layer of gold (0.02 µm). Then adjusted the focus of the laser beam onto the substrate, and tested the stability of the system scanning two points with 250 µm interval for 9 h. The results indicated that the displacements of the two scan points in X and Y directions were both stable and consistent with each other, which verifies the stability of the scan light path.

3.3 Test of array signal consistency

At first, we accurately adjusted the scan laser beam onto the top of microcantilever 1 and 2, and then carried out the temperature excitation test after the output signals were stable. With the high-precision temperature controller (±0.01 °C), the environmental temperature of microcantilever array gradually increased from 22 °C to 28 °C, and the data curves were achieved (as shown in Fig.5, the two signal curves are shifted from the base point). The experiment results show that the deflections of the two cantilevers practically remain consistent with each other in the same temperature change. After the temperature increase by 6 °C, the deflection deviation of the two cantilevers is 6.5% (the differential signal 26 nm dividing the total deflection signal 400 nm). As the detection of biochemical reaction using micro-cantilever sensing technique is mainly about the intermolecular specific binding, therefore, if the specific binding information could be measured out accurately, the microcantilever array deviation within 10% would not affect the test results. As shown in Fig.6, the detection accuracy of the commercial VCSELs cantilever array sensor (Concentris, Switzerland) is also of this order of magnitude.

To further test the reliability of the array detection, the microcantilever array was placed in a small biochemical reaction cell and driven the solution with a peristaltic pump to simulate the actual biochemical reaction environment. Then adjusted the scan light path and collect the array deflection
data in natural environment for 8 h. As shown in Fig.7, the tendency of the two cantilever deflections always stayed the same along with the environment changes (temperature, solution flow, etc), which indicates the consistency of the cantilever array and proves the detection reliability of the microcantilever array system.

3.4 Detection of specific biochemical reaction

3.4.1 Reagents

Clenbuterol antigen (CLEN), anti-CLEN antibody and chloramphenicol antigen (CAP) were purchased from Agronomy and Biotechnology Institute, China Agricultural University, China. Thiol (HS-CH2-COOH), 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), and N-Hydroxysulfosuccinimide (NHS) were purchased from Sigma. Phosphate buffer (PBS, 4.0 g NaCl + 0.1 g KH2PO4 + 1.48 g Na2HPO4·H2O + 500 mL H2O), TPBS (PBS + 0.5% Tween-20), 98% H2SO4, 30% H2O2 were used in this experiment.

3.4.2 Antibody modification on cantilever array

First, microcantilever array was dipped into “piranhadip” (H2O2-H2SO4, 1:3, V/V) for 10 min at room temperature. After washed with deionized water, the cantilever array was moved into a vessel filled with 200 μg L−1 thiol solution of 0.1 M for 20 h at room temperature (vessel blocked). With native sulfydryl (−HS), the thiol was self-assembled on the microcantilever gold side through the formation of Au−S bond. Then the microcantilever array was washed with ethanol and deionized water in sequence, and put into the mixed solution of 100 μg L−1 0.2 M EDC and 100 μg L−1 0.05 M NHS for 1.5 h to activate the carboxyl of thiol. After washed with deionized water, the microcantilever array was placed into the capillary array modification platform (Fig.8), where cantilever 1 was modified with anti-CLEN antibodies while cantilever 2 was modified with nothing as a reference cantilever. Thereto, the interior diameter and external diameter of the capillary were designed to be 200–300 μm and 300–330 μm, respectively. In modification, one end of the capillary surrounded the cantilever and the other end inserted into the anti-CLEN antibody solution for 2 h. After removed from the capillary array modification platform and washed with TPBS, the microcantilever array was fixed into the biochemical reaction cell. Then PBS was flowed and the laser light path was adjusted for experiment.

3.4.3 Application of microcantilever array

Figure 9 shows the results of clenbuterol antigen-antibody specific reaction detected by microcantilever array. After 500 μg L−1 CAP antigen solution injected, both the two cantilevers produced a same slight deflection, which indicates the response may be caused by the environmental interferences such as temperature drift, changes of the solution refractive index and pH, etc; In addition, anti-CLEN antibody modified on cantilever 1 does not react with CAP antigen specifically. Next, when the response curves were stable, 10 μg L−1 clenbuterol antigen solution was added. It was found that the response signal of cantilever 1 modified with anti-CLEN antibody was significantly greater than that of cantilever 2 unmodified with anti-CLEN antibody. The phenomena indicate that the specific binding of clenbuterol antigen-antibody on cantilever 1 causes a significant surface stress change, and the environmental interferences may cause the
slight deflection of cantilever 2. So the response difference between cantilever 1 and 2 (reference cantilever) is the true deformation signal (38 nm) caused by clenbuterol antigen-antibody binding.

In microcantilever biochemical detection, the mechanism of cantilever surface stress change induced by antigen-antibody binding is currently considered to be hydrophilic or hydrophobic interaction, electrostatic interaction, hydrogen bonding, etc\cite{[16]}. As for clenbuterol detected in this paper, electrostatic repulsion is considered to be the main factor. Substituting the relevant parameters into formula (1), then we can calculate that the surface stress change of cantilever 1 is about 0.0184 N m$^{-1}$ when 10 µg L$^{-1}$ clenbuterol antigen reacting with anti-CLEN antibody modified on the cantilever.

**References**


