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pH Measurements with a Microcantilever Array-Based Biosensor System

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Abstract

In this paper, we present a pH measurement method that uses a microcantilever-array-based biosensor system. It is composed of microcantilever array, liquid cell, micro syringe pump, laser diode array, position sensitive detector, data acquisition device, and data processing software. Four microcantilevers are functionalized with pH-sensitive MHA(mercaptohexadecanoic acid) as a probe, while three microcantilevers are functionalized with HDT(hexadecane thiol) as reference. We prepare PBS(phosphate buffered saline) solutions of different pH and inject them into the liquid cell with a predefined volumetric speed at regular time intervals. The functionalized mircocantilevers in the liquid cell deflect as a self-assembled monolayer on the microcantilever binds with probe molecules in the solution. The difference in deflection between the MHA-covered probe microcantilever and the HDT-covered reference microcantilever was used to compensate for thermal drift. The deflection difference clearly increases with increasing pH in the solution. It was shown that when the pH values of the PBS solutions are high, there were large variations in the deflection of microcantilevers, whereas there were small variations for low pH value. The experimental results show that the microcantilever array functionalized with MHA and HDT can detect pH value with good repeatability.

Keywords : pH, Microcantilever, HDT, MHA, Functionalization, Surface Stress

1. INTRODUCTION

When the size of a device is scaled down to micrometer scale, the surface stress is linearly proportional to the dimensions, while the surface to volume ratio increases with a decrease in the device dimensions. For this reason, the surface stress has a greater effect on MEMS (microelectromechanical system) structure and can induce deformation on the structure. Microcantilevers have shown excellent potential as sensors due to several notable advantages including ultrasensitivity, ease of mass production, and low cost. Microcantilever sensors have been applied to photothermal spectroscopy[1, 2], surface stress detection[3-7], infrared radiation detection[8], mass change detection[9-11], photothermal sensors[12]. Several studies have been carried out in order to recognize biomolecules by triggering the displacement of microcantilevers and measuring it by using self-assembly events such as receptor-reagent combination. Molecules for specific combination with bio-substances to be measured can be easily self-assembled on the microcantilever and make the surface functionalized. In a liquid containing target biomolecules, self-assembled molecules on the surface combine with the bio-molecules with the characteristics of anions. The combined bio-molecules show repulsive force to each other, thus producing stress on the functionalized surface stress and ultimately inducing bending of the surface. There are several ways to measure the bending of the surface to recognize the biomolecules. Recently, there have been many studies on applying cantilever deformation due to the surface stress to the measurement of bio-molecules. However, research on the influence of partial coating of different patterns on the microcantilever that can trigger different types of surface stress on the microcantilever has not yet been carried out.

The goal of this research is to find a method to sense minute changes in pH by a microcantilever array-based biosensor system. Microcantilevers partially coated with Au are prepared to measure the bending induced by surface stress and to find a good pH measurement method. The experiment was carried out with a biosensor system consisting of a cantilever array, fluid chamber, micro pump, laser diode and PSD(position sensitive detector) for measuring the displacement of the cantilevers. The surface of the microcantilevers was coated with different Au patterns, and half of the microcantilevers were

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functionalized with pH-sensitive MHA(mercapto hexadecanoic acid), while the other half of were functionalized with HDT(hexadecane thiol), both of which are not affected by pH. Then, we prepared two PBS solutions of pH 4.3 and pH 8.6, respectively, and injected them into fluid cell with a set volumetric speed at regular time intervals. Experimental results show that fully coated microcantilevers with Au on the surface have the greatest displacement. In case of the fully coated cantilever array, the measured average displacement was approximately 20 nm for PBS solution with pH 4.3, and 60 nm for PBS solution with pH 8.6. The microcantilevers also show good reproducibility in pH detection in this pH measurement experiment.

2. BENDING DEFORMATION MEASUREMENTS OF DIFFERENT GOLD-PATTERN-COATED CANTILEVERS

2.1 Experimental Setup

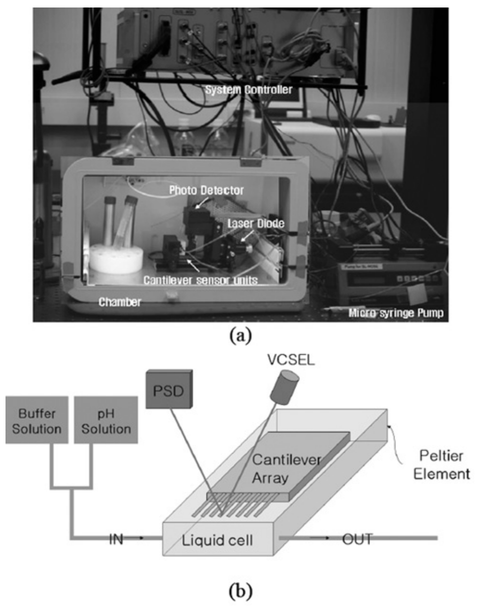

Fig. 1. (a) Multi-cantilever biosensor system setup, (b) Schematic diagram of multi-cantilever biosensor system.

Fig. 1(a) shows the biosensor system consisting of a liquid cell in which microcantilever array is installed, a signal processing unit to convert and store measurement signals, a control device unit controlling items such as the micro syringe pump and sample port, an insulation chamber box, and a computer with software installed to control the sensor system to process and analyze the measurement signal. Fig. 1(b) shows the schematic diagram of the biosensor system that can detect 8 different bio-substances using 8 micro-cantilever arrays. The test sample solution supplied through the sample port is flown into the liquid cell where the microcantilever array is installed and the deflection of the microcantilevers is measured using 8 laser diodes(VCSELs) and a photodetector. The measured data is saved to the computer through data acquisition device. The temperature of the liquid cell in which the cantilevers are installed is kept consistent with a thermocouple, and the temperature fluctuation was minimized by using an insulation box.

2.2 Functionalization of Partially Au Coated Microcantilever Array

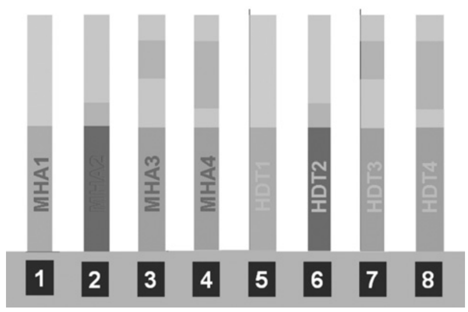

Fig. 2. Partially Au-coated microcantilever array.

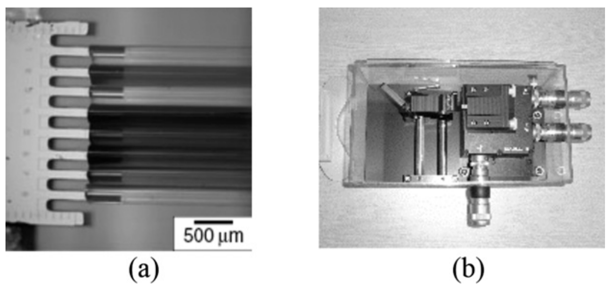

Fig. 3. (a) Functionalizing cantilever, (b) Functionalization device.

The cantilever array coated with thin layers of Ti/Au(3 nm/20 nm) have thickness of 1 ㎛, length of 750 ㎛ and width of 100 ㎛. Fig. 2 shows the cantilever array with four different Au patterns on the surface. In order to detect the cantilever deflection due to surface stress, four probe cantilevers were functionalized with MHA solution, and four reference cantilevers were functionalized with HDT solution. Two PBS test solutions with pH 4.3 and pH 8.6 were prepared. The capillary-type functionalization unit was used to functionalize the micro-cantilever as shown in Fig. 3, and each cantilever can be functionalized with different substances at the same time, since each cantilever is inserted into separate glass microcapillary arrays for functionalization.

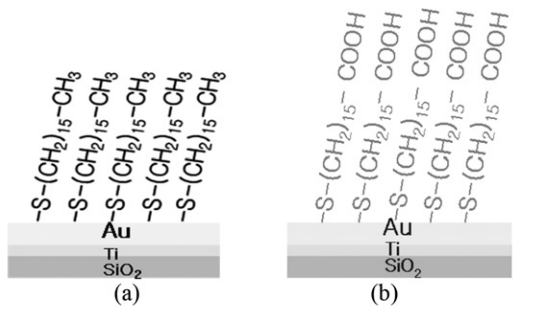

Fig. 4. (a) Functionalized reference cantilever with HDT, (b) Functionalized probe cantilever with MHA.

Fig. 4 shows the molecular formula of the SAM(self-assembly monolayer) for the functionalization of cantilevers. MHA enters a state of being neutral or having a lot of hydrogen ions (H^+) when pH is 5 or less, and enters a state of having a lot of negative carboxy group(COO-) ions as the hydrogen ions(H^+) get dissociated for pH of 5 or greater. The negative ions of the carboxy group repulse each other, thus the cantilever bends as shown in Fig 5 when the pH becomes higher than 5. Since the HDT does not perform unspecific bonding due to its low surface energy and unreactiveness, it is used as reference for eliminating the surrounding noise.

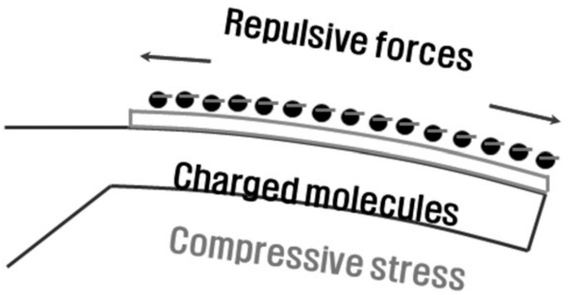

Fig. 5. Cantilever bending mechanism due to specific bonding.

Also, four microcantilevers with different patterns of Au coating were used to find the microcantilever with the most deformation in specific pH conditions.

2.3 Measurements Results

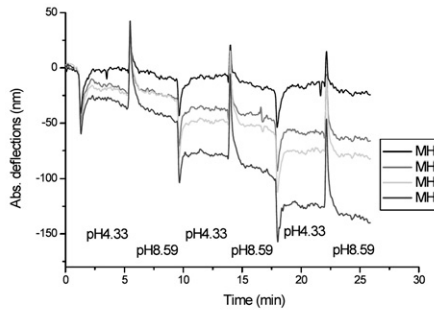

Fig. 6. Absolute displacement of partially Au-coated microcantilever array.

Fig. 6 shows the measurement results of absolute displacements of microcantilevers coated with Au and functionalized with MHA. MHA1 microcantilever had the surface fully coated with Au and showed the largest displacement. MHA2 microcantilever showed the second largest displacement, and MHA4 microcantilever showed the smallest displacement.

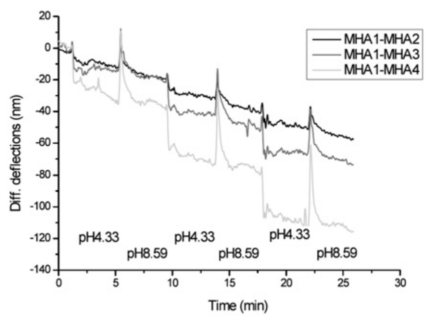

Fig. 7. Differential displacements of partially Au-coated microcantilever array.

Fig. 7 shows the displacement difference between MHA1 and all other microcantilevers. The displacement difference was largest between MHA1 and MHA4, and smallest between MHA1 and MHA2.

These experimental results show that the fully coated microcantilever is most sensitive in pH measurements. Among the partially coated microcantilevers, the microcantilever that has Au coating on the area of the support position of the microcantilever shows most bending.

3. pH MEASUREMENTS WITH FULLY AU-COATED CANTILEVER ARRAY

3.1 Functionalization of Fully Au Coated Cantilever Array

Four probe microcantilevers fully-coated with Au were functionalized with MHA solution, and three reference cantilevers fully-coated with Au were functionalized with HDT solution. Also, two PBS solutions with pH 4.3 and pH 8.6 were prepared.

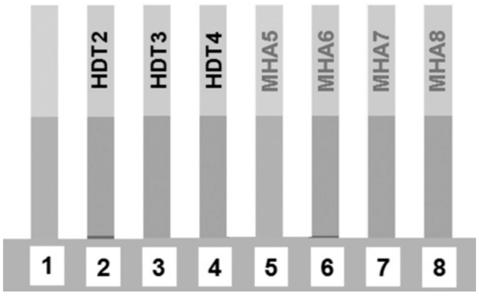

Fig. 8. Partially Au-coated microcantilever array.

For the measurements of pH, PBS solution with pH of 4.3 was inserted into the liquid cell with a volumetric flow rate of 5.0 ul/min for 10 seconds using a microsyringe pump. The bending was monitored for 190 seconds until the PBS solution with pH 8.6 was introduced and monitored for another 190 seconds.

3.2 Measurement Results

In Fig. 9, we can see that the magnitude of absolute displacement of the probe microcantilevers is smaller in case of pH 4.3 than in case of pH 8.6. But, the magnitude of absolute displacement of the reference microcantilever is negligible in both cases after the inserted test sample becomes stabilized.

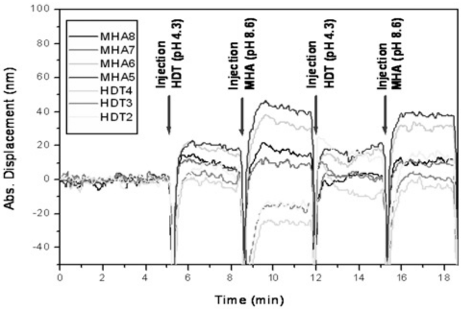

Fig. 9. Absolute displacement of fully Au-coated microcantilever array.

Fig. 10 shows the deflection difference between probe microcantilevers and reference microcantilevers used to eliminate thermal drift noise. From the result of Fig. 10, we can see more actual displacement of the microcantilevers due to pH change. Fig. 11 shows the averaged differential displacement (HDT(i)-MHA(j)). The measured average displacement was approximately 20 nm for the PBS solution with pH 4.3, and 60 nm for the PBS solution with pH 8.6. The averaged displacement difference of (HDT(i)-HDT(j)) and (MHA(i)-MHA(j)) shows almost zero displacement difference. This means that the reference and probe microcantilevers show uniform behavior for a test solution. With these results, it was shown that the fully coated Au microcantilever-based biosensor system provides reproducible results in pH measurement.

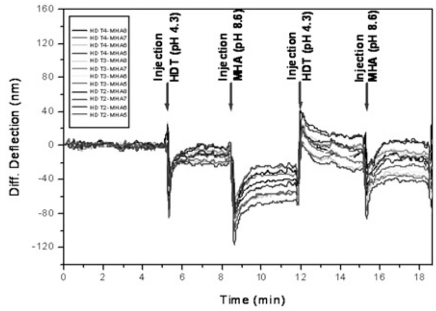

Fig. 10. Differential displacement of fully Au-coated microcantilever array.

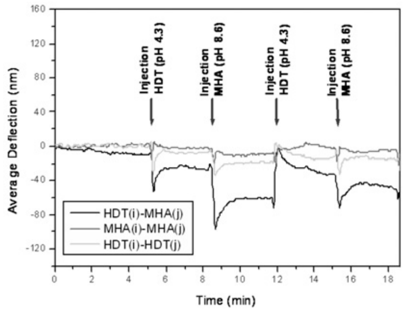

Fig. 11. Average differential displacement of fully Au-coated microcantilever array.

4. CONCLUSIONS

We prepared a microcantilever array biosensor system to prove the effectiveness of surface stress measurement as a pH measurement method. Different Au patterns were coated onto the microcantilevers, and experimental results show that the fully Au-coated microcantilever array deforms most, and microcantilevers partially coated on the supporting areas produce the next largest displacement. The measured average displacement was approximately 20 nm for PBS solution with pH 4.3, and 60 nm for PBS solution with pH 8.6. Also, it was shown that the fully coated Au microcantilever-based biosensor system provides reproducible results in pH measurement.

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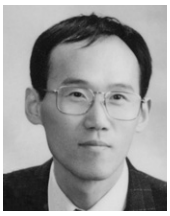

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