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Fabrication and characteristics of microcantilever-based biosensor for detection of the protein-ligand binding

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Abstract. This paper describes a proposal for a microcantilever-based biosensor that can be used in investigating the adsorption characteristics of protein-ligand binding on a silicon nitride/gold coated surface. We have detected streptavidin-ligand binding using this microcantilever detection system. The microcantilevers can be mass-produced by a conventional surface micromachining technique. This technique has advantages of cost efficiency, simplicity, and the ability to be fabricated in an array. A transparent fluid cell system, where a gold coated microcantilever was mounted for the injection of bio-molecular solution, was fabricated using polydimethylsiloxane (PDMS) and fused silica glass. The microcantilever was deflected as a result of the difference of surface stress caused by the formation of the self-assembly monolayers (SAMs) of biomolecules on the gold coated side of the microcantilever. The sequential specific interactions of cystamine dihydrochloride/glutaraldehyde/streptavidin were detected by both optical and electrical methods. We confirmed that the deflections were induced by biomolecular adsorption on the gold coated microcantilever. This study proved to be applicable to real-time monitoring of biological interactions such as specific DNA sequences, proteins, and so on.

1. Introduction
Microcantilevers, typically used in commercial atomic force microscopy (AFM), have been developed as sensitive stress sensors. In recent years, enzyme-linked immunosorbent assays (ELISAs) have been widely used for the detection of specific biomolecular interactions. However, this approach required fluorescent tag labeling of samples, necessitated high analytical skills, and was expensive and time-consuming. To alleviate these disadvantages, several label-free methods were developed such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM), scanning tunneling microscopy (STM), AFM, and more. Self-assembly has been one of the most important properties of biomolecules. The self-assembly principle has been utilized with numerous DNA hybridizations or antigen-antibody reactions. In recent years, this principle has largely been applied to memory devices and bio-medical fields. These interactions can collectively produce the mechanical motion of the microcantilever. These principles have also been adapted to elucidate the microcantilever deflection created by protein-ligand binding. We present a microcantilever-based biosensor for the analysis of biochemical reactions between a protein and a ligand. The mechanical response of the microcantilever can be monitored as a deflection of the microcantilever with respect to a change in the surface stress on the protein-ligand binding side of the microcantilever relative to the other side. The quantities of bending were measured using both optical and electrical detection methods. The optical beam deflection method has been the most widely used technique to measure the deflection of cantilevers. It has sub-angstrom resolution

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and can be easily operated. The electrical detection method included integrated piezoresistive readout which greatly facilitated operation in solutions since the refractive indices of the liquids did not influence detection. Thus, measurements could be performed even in non-transparent liquids [1,2]. The microcantilevers were fabricated through a surface micromachining technique that had attractive advantages in terms of cost efficiency, simplicity and ability of fabricating in an array. A bottom-up method, a surface micromachining technique was widely used in the micro electro mechanical system (MEMS) in comparison with the bulk micromachining of a top-down method [3,4]. The proposed microcantilever-based biosensor [5,6] utilized silicon nitride as the main substrate material.

In this work, for detection of biomolecules, the deflections of the microcantilever were measured by both an optical method and an electrical method. The microcantilever detection system required a small quantity of the solution and thus a provided fast reaction time. In addition, it was advantageous in terms of miniaturization, portability, and the array design of the devices.

2. Experimental Details

2.1 Fabrication of piezoresistive microcantilever

The fabrication process of the piezoresistive microcantilever is illustrated in Figure 1. Amorphous silicon with the thickness of 2 µm, was deposited on a thermally oxidized layer as a sacrificial layer with low-pressure chemical vapor deposition (LPCVD). A 300 nm-thick polycrystalline silicon (poly-Si) layer was deposited on a 300 nm-thick LPCVD silicon nitride as a piezoresistive material using LPCVD. The ion implantation was performed on a poly-Si layer. A doped poly-Si layer was further processed by lithography and ICP dry etching. A silicon nitride film was deposited under a low stress condition to eliminate any curling distortion. Since the thermal processing considerably affected the mechanical properties of the cantilever, silicon nitride was further processed by lithography and magnetically enhanced using a reactive ion etching technique (MERIE). The metallization was performed with piezoresistive material to pad the microcantilever for the recognition of the change in resistance of the piezoresistive material integrated in the microcantilever. A contact hole was formed by the MERIE and was filled with aluminum by a sputtering technique. The Cr/Au (3 nm/30 nm) layer was deposited on the upside surface of the microcantilever by sputtering then lifted it off. Finally the sacrificial layer was removed by XeF2 dry etching to release the microcantilever.

Figure 1. The schematic fabrication process flow of the piezoresistive microcantilever for optical and electrical measurements

2.2 Principles of protein-ligand binding

We investigated the protein-ligand binding process on the gold coated microcantilever. The microcantilever was positioned in a fluid cell system. Cystamine dihydrochloride, glutaraldehyde, and streptavidin solutions were sequentially allowed to flow over the microcantilever. The major principle in gold/cystamine dihydrochloride/glutaraldehyde/streptavidin binding formation is the self-assembly concept. Initially, cystamine dihydrochloride was injected into the system to facilitate its interaction with a gold forming self-assembled monolayer (SAM). The interfacial contact of gold with cystamine dihydrochloride was the alkanethiol group (-SH) located at the terminal amine. Gold was previously
reported in numerous papers for its known interaction with different compounds like cystamine dihydrochloride in this study. The glutaraldehyde solution, which contained terminal aldehyde groups, was injected next over the microcantilever. One aldehyde group formed a covalent bond with the amine group of cystamine dihydrochloride previously immobilized on the gold surface while the other aldehyde group bonded with streptavidin. According to these principles, SAMs of gold-cystamine dihydrochloride-glutaraldehyde-streptavidin were formed consecutively. The microcantilever deflection was caused by the protein-ligand binding process on the binding site.

2.3 Measurement set-up
All reagents used in this study were purchased from the Sigma-Aldrich Company. An optical detection method using a CP AFM (Digital Instruments, Santa Barbara CA, USA) was utilized for the optical laser beam deflection system as illustrated in Figure 2. In the electrical detection method, the change in resistance was determined by a constant voltage supply and the real time measurement of current values is as depicted in Figure 3. The fluid cell system for the introduction of sample solutions was fabricated using a PDMS and fused silica glass with a UV-ozone cleaner. The flow rate of the liquid was maintained at 10 ml/hour. For system stabilization purposes, the deionized water was first allowed to flow for at least 30 minutes prior to the measurement.

3. Results and Discussion
In the optical detection method, the extent of the deflection (A-B voltage difference values of Position Sensitive Photo Diode) such as a bending-up or bending-down of the microcantilever was measured. On the other hand, the electrical detection method determined the change in electrical conductivity of a piezoresistive material, such as poly-Si, when subjected to strain.

Figure 4 demonstrates the result of the optical detection method experiment of the 11.3 mg cystamine dihydrochloride/ml, 0.04 mg glutaraldehyde/ml and 0.5 mg streptavidin /ml solutions. In order to have a point of reference, deionized water was allowed to flow for about 30 minutes at the beginning of the experiment. This period of time did not show any significant change in the amount of deflection as shown by the stable baseline. After 30 minutes, the cystamine dihydrochloride solution was injected which caused instant deflection. After 70 minutes from the injection of the cystamine dihydrochloride solution, the glutaraldehyde solution was injected and a deflection was also observed. The streptavidin solution was injected at 190 minutes which lead to a higher extent of deflection as signified by the further increase of the deflection unit value. These results indicated that the sequential SAM formation of gold and cystamine dihydrochloride followed by protein-ligand binding took place. The amount of deflection changed corresponding to each injection time because of the bending of the microcantilever.

Figure 5 illustrates the result of the electrical detection method experiment using the same solution concentrations mentioned previously. After each separated injection of cystamine dihydrochloride, glutaraldehyde, and streptavidin solutions, a decrease in the resistance values was recorded. This signified that the resistance value of the piezoresistive material (poly-Si) changed with respect to the
deflection of the microcantilever caused by the sequential binding of cystamine dihydrochloride, glutaraldehyde, and streptavidin on the gold coated side of microcantilever. After a stabilization period of about 50 minutes, the cystamine dihydrochloride solution was injected and the resistance value was decreased down to about 1.2 kΩ. A further decrease in resistance was observed when the glutaraldehyde solution was injected after 150 minutes, with a resistance value of 127 kΩ. The final injection of the streptavidin solution at 250 minutes rendered a further decrease of 1 kΩ resistance.

Figure 4. The deflection curve in the optical detection method with respect to the deflection of the microcantilever because of the protein-ligand binding

Figure 5. The resistance variation curve in the electrical detection method with respect to the deflection of the microcantilever because of protein-ligand binding

Both detection methods presented were able to successfully reveal the behavior of the biosensor in response to the SAM formation and protein-ligand binding process. But the electrical detection method was more amenable since it involved a simpler setup as compared to the former which involved a laser detection system. Thus, the preference of detection method depended on the applications involved.

4. Conclusions
The piezoresistive microcantilever for the biosensor was fabricated using the surface micromachining technique for the detection of the protein-ligand binding. The surface micromachining technique had attractive advantages in terms of cost efficiency and simplicity in comparison with the bulk micromachining technique. This technique was successful in detecting the cystamine dihydrochloride, glutaraldehyde and streptavidin. Self-assembled protein-ligand binding was investigated using the piezoresistive microcantilever with both the optical and electrical detection method. It was verified that the deflections of the microcantilever detected various bio-molecules and that microcantilever was suitable for a biosensor.

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