Development of a biosensor based on laser-fabricated polymer microcantilevers

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We develop high-sensitivity biosensors based on microcantilevers. The polymer microcantilevers are fabricated by fast and cost-effective laser machining processes. Polymer film is selected because it gives better sensitivity of deflection measurements than silicon due to its lower Young's modulus and also its cost is much lower. We demonstrate using these polymer microcantilevers for biological molecular analysis in a DNA hybridization experiment. It is shown that our biosensor is capable of detecting 12 base oligonucleotide with concentrations as low as 0.01 μM. © *2004 American Institute of Physics*. [DOI: 10.1063/1.1791731]

Biosensors detect and transmit information regarding a physiological change or the presence of various chemical or biological materials in the environment. Many types of biosensors have been developed since the demonstration of the first biosensor-enzyme electrodes in 1962.[1] Most biosensors are constructed based on electrochemical transducers, but a number of other types are growing in importance, which are based on optical, piezoelectric, surface acoustic waves, and thermal effects.[2] Biosensors using microcantilevers have attracted considerable interest in the last few years.[3,4] These sensors are able to detect single-strand DNA hybridization and protein-ligand binding,[5–8] gaseous analytes,[9,10] *p*H variations,[11,12] and protonization.[13] The microcantilevers transduce the recognition event from their receptor-coated surface into a mechanical deflection. Upon binding of the ligand to the receptors, the adsorption stress leads to bending of the cantilever toward or away from the receptor side depending on the nature of the chemical bonding of the molecules. The deflection of microcantilever can be measured using the optical beam deflection technique, which is highly sensitive and widely used in atomic force microscopy. As such, high sensitivity sensing becomes possible.

The defection δ, of a cantilever is caused by the surface stress difference of the top (receptor-coated) and the bottom surfaces. The amount of deflection δ can be estimated according to the Stoney's formula:[14]

$$\delta = \frac{3(1-\nu)(\sigma_1-\sigma_2)L^2}{Ed^2}, \tag{1}$$

where ν is the Poisson ratio of the cantilever material; σ_1 and σ_2 represent the surface stress of the top and bottom surfaces, respectively; L and d are the length and the thickness of the cantilever, respectively; and E is the Young's modulus of the cantilever material.

For the cantilever biosensors reported in the literature, silicon and silicon microfabrication techniques are commonly used. In our work, we propose to use polymer films as a cheaper alternative as the cantilever material and to use laser machining techniques to fabricate polymer cantilevers. As can be seen from Eq. (1), the amount of deflection is inversely proportional to the Young's modulus. With a much smaller Young's modulus (about 1/20 of that of silicon), polymer cantilevers have the potential of obtaining much higher sensitivity compared with the commonly used silicon cantilevers.

Our microcantilevers are fabricated using 6-μm-thick polyethylene terephthalate (PET) films (Goodfellow, Inc.). PET can be easily machined using the UV laser ablation technique, which is a fast, clean and cost-effective process comparing to traditional silicon microfabrication techniques. The mechanisms of UV laser ablation of polymer have been extensively studied. Generally, photochemical fragmentation and thermal reaction are involved when a UV laser is used.[15] In this work, a KrF excimer laser ($\lambda = 248$ nm, pulse width $= 26$ ns) is used as a laser source to machine polymers. A laser fluence of 1.6 J/cm^2 and a pulse repetition rate of 5 Hz are used. At this laser fluence, the ablation rate is about 0.9 μm/pulse. The laser beam irradiates onto the PET surface with a diameter of 50 μm. The PET film is mounted on a computer-controlled x-y stage which has a 0.1 μm resolution, and its moving speed is set to be 10 μm/s. The stage moves following predesigned paths according to AutoCAD files containing cantilever patterns. The total time for laser fabrication is only a few minutes. With the use of an industrial type of excimer laser, machining speed can be much improved. Details of excimer laser machining of various microstructures have been described elsewhere.[16]

Figure 1 shows the image of a laser-fabricated three-cantilever array. Each cantilever is 600 μm long and 250 μm wide. The base area is machined for the purpose of mounting. A gold film of 50 nm thickness is evaporated on

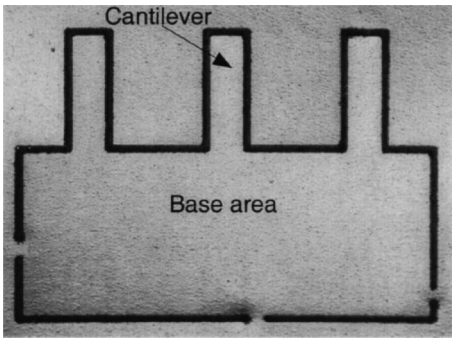

FIG. 1. Image of a three-cantilever array made with the laser microfabrication technique (cantilever dimension: width 250× length 600× thickness 6 μm).

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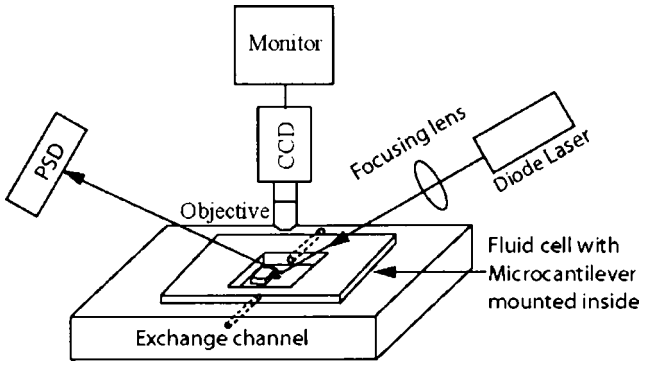

FIG. 2. Experimental setup of the biosensor including a fluid cell, optical beam deflection measuring system, and CCD monitoring system.

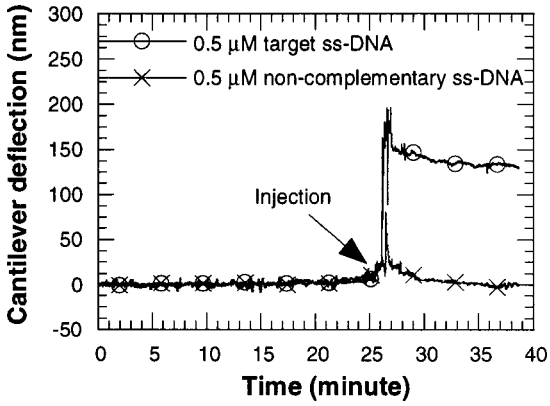

FIG. 3. Detection of microcantilever deflection induced by DNA hybridization (hand injections of 0.5 μM noncomplementary ss-DNA and 0.5 μM target ss-DNA, injected volume 0.2 mL).

one side of the cantilever freshly after the laser machining for DNA immobilization.

The biosensor system we developed in this work includes a fluid cell inside which the microcantilever is mounted, a syringe or a syringe pump, an optical beam deflection measuring system, and a charge-coupled-device (CCD) monitoring system, as shown in Fig. 2. The whole detection system is mounted on an optical table to reduce vibration. A cubic box made of acrylic is used to cover the biosensor to prevent disturbance from the air flow and background light. The base of the sensor system is made of brass to improve stability. The fluid cell is made of acrylic, which is inert to chemicals involved in our experiments. The monitoring optics is basically a microscope system, which includes a black/white CCD (SY-VCB3524, SANYO), a 10 $\times$ objective lens (Olympus, Inc.), and a television monitor. The optical resolution of the monitoring system is about 5 μm, which is sufficient for helping to direct the laser beam onto the cantilever. The polymer cantilever is mounted in the chamber using adhesives. A diode laser is focused on the cantilever and the reflected laser beam is aligned onto a position-sensitive detector (PSD) for the deflection measurement.

We perform DNA hybridization experiments to demonstrate applications of the polymer biosensor. The probe DNA is a thiolated single-strained DNA and is absorbed on the side of cantilevers coated with gold. It is a 12 base oligonucleotide with the sequence of 5′-HS-(CH_2)6-TGC ACT AGA CCT-3′, abbreviated as HS-ssDNA. The thiolated modification enables covalent binding to the gold-coated surface. The complementary DNA used as the target DNA is an ss-DNA with the sequence of 5′-AGG TCT AGT GCA-3′. A noncomplementary ss-DNA with the sequence of 5′-TGC ACT AGA CCT-3′ is used as a reference. The DNA used in this study is synthesized by standard phosphoramidite chemistry and is purchased from Integrated DNA Technologies (Coralville, IA). Cantilever functionalization is prepared before the DNA hybridization experiment by inserting the fresh coated cantilevers into a reservoir which is filled with 10 μM solution of HS-ssDNA in 1.0 M potassium phosphate for around 2 h at room temperature. Functionalized cantilevers are stored in a refrigerator at 4°C. They are equilibrated for several hours in the liquid cell to reach room temperature before use.

DNA hybridization is performed in a saline sodium citrate hybridization buffer—5$\times$ SSC buffer (Calbiochem, La Jolla, CA) at room temperature. The functionalized cantilever is mounted inside the liquid cell and the target DNA is injected into the cell using a syringe (by hand injection or by a syringe pump). Nonspecific binding, *p*H value, polymer–liquid reaction, as well as polymer swelling, could induce signal drift. In order to detect the cantilever deflection caused by DNA hybridization only, the target DNA is injected after a stable signal is obtained, which typically takes several hours. The experiment proceeds as follows. First, the liquid cell is filled with the 5$\times$ SSC buffer. After deflection is stabilized, the target DNA in the 5$\times$ SSC buffer solution is then injected. The PSD signal changes are recorded during the whole hybridization period using a computer data acquisition system. Only one of the cantilevers in the cantilever array is monitored since our current sensing system has only one light source and one PSD.

The environmental and electronic noises of the sensor system are found by monitoring reflection from a probe DNA-coated cantilever mounted in the fluid cell filled with the 5$\times$ SSC buffer. The data show that the noise level of the cantilever in the fluid cell is about 2 nm. Although the optical system is able to measure cantilever deflection of less than 1 nm, the detection limit is set by other factors such as temperature fluctuation and fluid motion.

Cantilever deflection as a function of time for the target ss-DNA is shown in Fig. 3. For the experiments shown in Fig. 3, the injection is done by hand which generally causes some disturbance (a spike) to the signal, as shown in Fig. 3. The liquid with a total volume of 0.2 mL is injected within 10 s, causing a fast response in the deflection signal, as shown in Fig. 3. The final deflection of the cantilever is 122 nm for the DNA concentration of 0.5 μM. According to the calibration, the increased deflection indicates that the cantilever bends downward, away from the DNA-coated side (the probe DNA is coated on the gold film on the top of the cantilever). To confirm that the deflection signals are caused by DNA hybridization, the buffer containing 0.5 μM noncomplimentary ss-DNA is also injected. As shown in Fig. 3, there is no deflection produced after injection.

In the experiments, it is found to be difficult to detect target ss-DNA with a concentration lower than 0.1 μM when the total injected volume is 0.2 mL; the cantilever deflection caused by DNA hybridization is close to the noise level at lower concentrations. In order to increase the detection resolution, a larger volume of target ss-DNA is injected at a slow and constant speed using a syringe pump. It is thought that

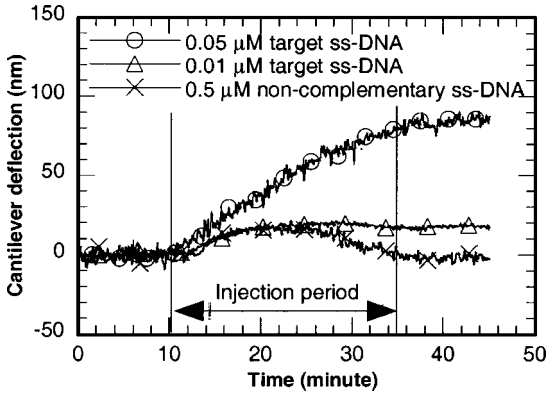

FIG. 4. Detection of microcantilever, deflection induced by DNA hybridization (syringe pump injections of 0.5 μM noncomplementary ss-DNA, target ss-DNA with concentrations of 0.05 μM and 0.01 μM, injected volume 1.67 mL).

more target ss-DNA would bind to the probe DNA if a larger volume of the target ss-DNA solution is continuously injected. Therefore, deflection can be detected at lower DNA concentrations. In this work, the volume flow rate of the syringe pump is set at 4 mL/h, and a total volume of 1.67 mL solution is injected into the fluid cell in 25 min. The results of DNA hybridization at DNA concentrations of 0.05 and 0.01 μM are shown in Fig. 4. The resulting cantilever deflections are 85 and 18 nm for 0.05 and 0.01 μM, respectively. For comparison, the 0.5 μM noncomplementary ss-DNA is also injected. A fluctuation the deflection of about ±5 nm is observed which is caused by the syringe pump, but the mean deflection remains zero. A larger disturbance is induced when larger injecting speeds are used.

The experimental procedures are repeated for a number of target ss-DNA concentrations. The results are summarized in Fig. 5. Comparing the results obtained from biosensors made of silicon cantilevers,[5] which reported a lowest concentration of the target ss-DNA of 0.08 μM, our biosensor is able to detect lower concentrations (0.01 μM). We believe it is possible to further improve the sensitivity of our biosensors. Methods for improving a cantilever's sensitivity are implied by Eq. (1). For instance, larger deflection could be produced by increasing the cantilever length or reducing its thickness while keeping other parameters the same. The main challenge for a thinner cantilever is to overcome the cantilever stability problem because the thinner cantilever is more liable to the flow disturbance. Another method for reducing the noise is to use a reference cantilever for monitoring the disturbance from the environment such as temperature fluctuation and flow motion.[5,8] Various techniques for improving sensitivity are currently under development.

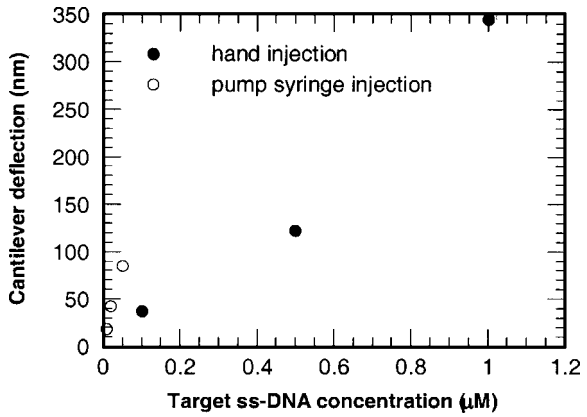

FIG. 5. Detection of microcantilever deflection induced by different target ss-DNA concentrations.

In conclusion, we developed a highly sensitive biosensor based on polymer microcantilevers which are fabricated using laser micromachining techniques. In DNA hybridization experiments, we showed that our biosensor is capable of detecting 12 base oligonucleotide with concentrations as low as 0.01 μM, higher than that reported in the literature. Therefore, polymer cantilevers, combined with the laser fabrication techniques can be a viable alternative to silicon-based sensors and surface machining techniques.

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