Microcantilever Array Sensors for Biomolecular Detection

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ABSTRACT

Microcantilever based sensors are a new class of extremely sensitive sensor device that is currently being developed for chemical and biological detection. In the work described here, Oligonucleotide probes for DNA and mRNA were immobilized on the gold coated side of silicon microcantilevers via thiol linkers and exposed to complementary and non-complementary sequences using microliter volumes of sample solutions. The surface stress generated by specific recognition between complementary sequences could be monitored as it induced a deflection of the cantilever. The deflection response of up to sixteen cantilevers could be read in parallel using a new optical reader based on an array of laser diodes (VCSELs). As a model system for affinity binding, biotin-streptavidin interaction was also monitored against unspecific streptavidin adsorption

Keywords: microcantilevers, biosensor arrays, DNA hybridization

1 INTRODUCTION

Microcantilever sensors are a new class of extremely sensitive device, currently being developed for chemical and biological detection [1-5]. Microcantilevers transduce recognition events on their receptor-coated surfaces into mechanical deflections. The deflection of the microcantilever that is of the order of a few nanometers, can be measured using an optical beam deflection technique. Differential surface modification is an essential feature for the functioning of cantilever-based sensors. The present work is motivated by the need for novel technologies for simultaneous detection of multiple species of biological importance that can contribute to early detection of genetic diseases or to fast drug screening. DNA mutations are known to cause a variety of diseases and there is a great demand for technologies that can detect single point mutations with high accuracy and speed. Microcantilever array based detection offers the potential for high throughput detection.

Monitoring of hybridization of DNA has been reported using other techniques like quartz crystal microbalance [6], electrochemical techniques [7], and surface plasmon resonance spectroscopy [8]. Although these techniques can be used without the use of molecular labeling, the microcantilever sensor platform has the unique advantage that simultaneous detections can be carried out in parallel. The overall goal of this sensor development is to achieve such capabilities. In the following we present preliminary results obtained by monitoring the deflection response of an array of sixteen cantilevers coated with ssDNA probes and biotin due to specific and unspecific molecular adsorption.

2 MATERIALS AND METHODS

2.1 Cantilever array reader

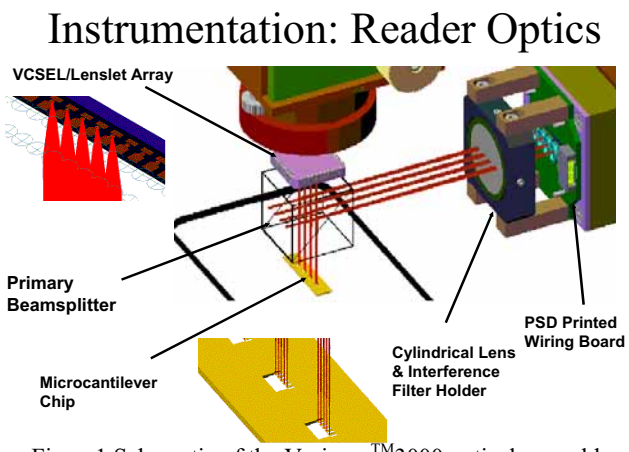

Figure1 Schematic of the Veriscan™3000 optical assembly. In the configuration used here, four groups of four microcantilevers are monitored, plus one laser in each grouping (or assay well) monitors a reference plane.

A commercial microcantilever reader and fluidics system was used in these experiments (Veriscan™3000, Protiveris, Inc). Figure 1 shows a schematic of the optical assembly In this instrumentation the light source is a

VCSEL chip with 64 powered lasers. In the configuration employed here, sixteen lasers are used to monitor cantilever deflection and four are used to monitor reference planes for removing non-cantilever signals (such as refractive index changes and temperature induced movement of the opto-mechanical assembly). An array of four position sensitive detectors (PSDs) track the deflections of the lasers.

2.2 Cantilevers and Chemicals

Array chips consisting of 16 rectangular cantilevers plus four reference mirrors divided into four wells (Protiveris Inc.) were used. The fluidic system allows each well to be addressed independently. It is therefore possible to treat each well independently, thus immobilizing different molecules.

All cantilevers in the array are identical: rectangular in shape (dimensions 500 µm x 150 µm x 1 µm), made of single-crystal silicon and with a nominal spring constant of 0.04 N/m. Cantilevers were coated on one side with 30 nm of gold with a 5 nm Ti/W adhesion layer.

The ssDNA probe used was a 25-mer oligonucleotide with a thiol group at the 5' end (Synthegen, USA). The base sequences used are:
Probe:
5'-HS $(CH_2)_6$GGCCATCGTTGAAGATGCCTCTGCC-3'
Complimentary Target:
5'-GGCAGAGGCATCTTCAACGATGGCC-3'
Non-compl. Target:
5'-GATTAGAGTCCCGCAATTAATCATT-3'
The immobilization buffer used was a 1M KH_2PO_4 at pH 3.8. The hybridization buffer used was a solution of 150 mM NaCl, 20 mM Na_2HPO_4, EDTA 0.1 mM, Tween 20 0.005%, pH 7.4. A blocking solution of mercapto hexanol, (MCH), 1 mM in water was used to remove any weakly bound probes and to block any vacancies on the gold surface. The HS-PEG-Biotin (Polypure Inc., Norway), HS-PEG-OH (Toronto Research Chemicals Inc., Canada), and Streptavidin (Pierce Chemicals, USA) were commercially available.

2.3 Cantilever Functionalization Procedures

Cantilevers were ozone cleaned prior to use, followed by several rinsing steps with deionized water. The cantilever array chip was then mounted inside the flow cell. The immobilization of the thiolated probes was carried out in-situ and we could monitor the resulting changes in cantilever deflection.

HS-$(CH_2)_6$-ssDNA probe: The cleaned cantilevers were exposed to 1 µM probe solution for forty minutes for immobilization. This step was followed by flowing deionized water through the flow cell for 20 minutes. Then 200 µL of 1 mM MCH in water was allowed to flow through the flow cell for 20 minutes followed by flow of water and hybridization buffer to remove unbound MCH. This results in cantilever surfaces with a mixed monolayer of strongly bound ssDNA and MCH.

HS-PEG-Biotin: Functionalization of the cantilever surface with HS-PEG-Biotin and HS-PEG-OH was carried out by exposing the cleaned cantilever surfaces to 1 mM solutions in 20 mM HEPES buffer, pH 7.4 for 40 minutes. Weakly bound species were removed by washing with buffer solution.

3 RESULTS

3.1 Thiol adsorption

We could follow the adsorption of probes in-situ. As an example, figure 2 shows the deflection response of the cantilevers during incubation with MCH. A net deflection response of about 1 µm after 30 minutes can be observed for all cantilevers. The MCH probably removes the weakly bound ssDNA from the gold surface and also fills any void spaces.

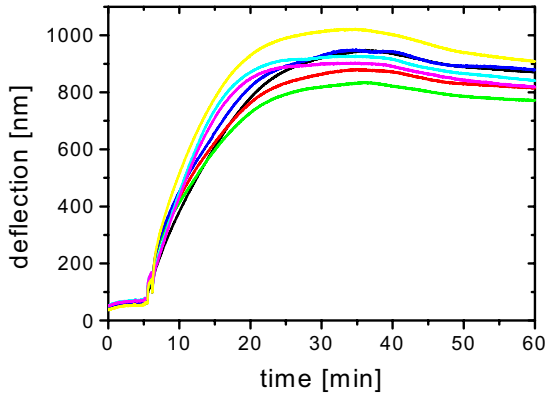

Figure2: deflection response of the array due to MCH thiol adsorption under continuous flow (10 µl/min). At t=5 the MCH solution enters the wells.

3.2 DNA hybridization results

The target solutions, at a concentration of 200 nM, were introduced into the flow cell via a 200 µL sample loop and a six port low pressure injection valve (Upchurch Scientific, USA) after baseline stabilization is attained. The deflection signals obtained upon introduction of complimentary and non complimentary target solutions are shown in Figure3. The difference signal between the complimentary and non complimentary deflections is 60 nm.

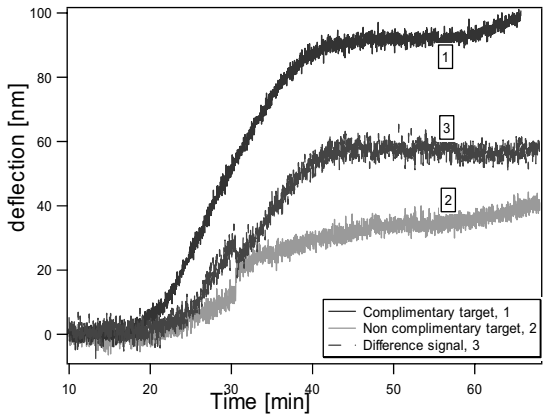

Figure3: Cantilever bending upon introduction of complimentary and non complimentary targets at a flow rate of 5 µL/min. The gold side of the cantilever is immobilized with HS-$(CH_2)_6$-ssDNA probe and MCH. A deflection of 60 nm was obtained upon hybridization.

3.3 Biotin Streptavidin Binding results

HEPES buffer was allowed to flow through the cell until a stable baseline was attained. Thereafter, 200 µL of 1.6 µM Streptavidin solution in HEPES buffer was introduced at a flow rate of 5 µL/min. The deflection signal obtained is shown in Figure 3. The net deflection observed as a result of streptavidin binding to the immobilized biotin was 450 nm.

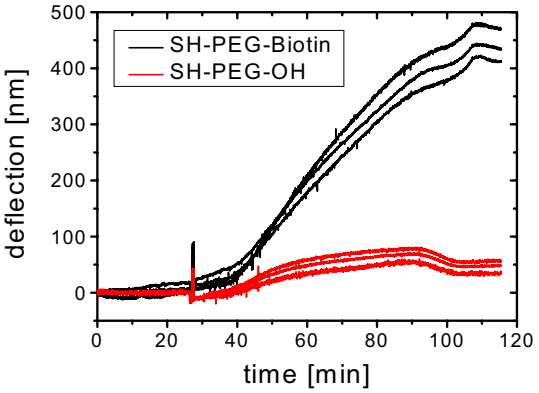

Figure4: Cantilever bending upon introduction of Streptavidin solution. The gold coated side of the cantilever surface is immobilized with HS-PEG-Biotin. The reference cantilevers were coated with HS-PEG-OH.

4 DISCUSSION AND FUTURE WORK

The results from the DNA hybridization and biotin streptavidin binding experiments show the capability of the microcantilever sensors to recognize specific interactions and transduce small energy changes associated with molecular binding events into detectable deflections. Reported results of DNA hybridization using similar silicon cantilevers (500 µm x 100 µm x 1 µm) with a spring constant of 0.02 N/m gave a deflection signal of 8.1 nm [5]. The higher values for the deflection that we obtained might be due the higher surface area of our cantilevers which results in a higher number of surface hybridizations events.

The capability to use arrays of cantilevers coated with different bioprobes and to monitor in parallel the response of the whole array opens the path to high throughput screening applications.

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