Design and Analysis of a high sensitive Microcantilever Biosensor for Biomedical Applications

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Abstract

Although becoming popular in label-free, rapid, and real-time assaying of biomolecules, the sensitivity of microcantilever biosensors decrease as the concentration of analytes decrease. The sensitivity of a microcantilever biosensor depends on its deflection that occurs due to interaction between the analyte and its bioreceptor molecule. Therefore, designing a cantilever biosensor which can assay analytes in low concentrations is important. In this study, we propose a new cantilever design that can detect and measure analytes in extremely low concentrations. By introducing a narrow strip towards the fixed end of the rectangular cantilever, we can reduce cantilever flexural stiffness and hence increase the deflections at its free end. A commercial finite element analysis software ANSYS is used to analyze and verify the proposed model. Results show the proposed cantilever bends nearly twice the conventional for same surface stress. This high sensitive design can significantly increase the sensitivity and range of cantilever biosensors. Furthermore, using a combination of conventional and proposed cantilevers array on same chip, a universal microcantilever biosensor can be designed that can assay analytes in large dynamic concentration range.

1. Introduction

Biosensors are devices that transduct biorecognition event into a measurable signal. A biosensor consists of two components: a biorecptor and a transducer. A bioreceptor is a biomolecule that combines with and hence recognizes the target molecule. Transducers convert this biorecognition event into a measurable signal. In a biosensor, the two components are integrated into a single sensor. For biosensing, labeling is a basic requirement for detecting and analyzing biomolecules and bioreactions. Labeling is however an expensive and time consuming process. The ability of label-free detection, scalability to allow massive parallelization, and sensitivity of the detection range applicable to *in vivo* problems are the important requirements for a future generation of biosensors [1]. Therefore, researchers are trying to develop label-free biosensing techniques. For label-free detection currently there are three popular candidates: surface plasmon resonance (SPR) [2], quartz crystal micro-balances (QCM) [3], and cantilever array biosensors [4,5]. SPR can analyze only one sample at a time. Moreover, detection of small molecules is its another limitation. Although more sensitive than SPR, QCM has limited scalability because its mass detection ability depends strongly on sensor surface. Compared with SPR and QCM, cantilever biosensors are more sensitive and through parallelization can achieve high throughput.

For biorecognition, one cantilever surface is made biosensitive by depositing a sensing layer onto it (Fig.1). This layer either contains the bioreceptors or the bioreceptors are covalently bonded to it. This process is known as functionalization. The reaction between an analyte and its bioreceptor molecule is unique. The most common forms of bioreceptors used in biosensing are based on proteins, antibody/antigen or nucleic acid interactions. As shown in Fig.1, when the analyte molecules are adsorbed onto the functionalized cantilever surface, surface stresses are generated that bend the cantilever. Wu et al. [6] reported that deflection may be upward or downward depending on the type of molecules involved. Since the cantilever deflection depends on the molecular species and its concentration, by measuring the cantilever deflection the attaching species as well as its concentration can be determined. The deflection depends linearly on molar concentration of the target analyte. McKendry et al. [5] used an eight cantilevers array to detect unlabeled DNA hybridizations at nanomolar concentrations within minutes. Arntz et al.

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[1] used a similar array for real-time detection of two cardiac biomarkers proteins myoglobin and kinase, whose level indicate presence of acute myocardial infarction, a heart disease. Further, they reported that the sensitivity of proteins detection by cantilever array technique is several orders of magnitude less than that for DNA hybridization reported in [6]. Zhang et al. [7] used microcantilevers in rapid and label-free detection of biomarker transcripts in human RNA in picomolar range.

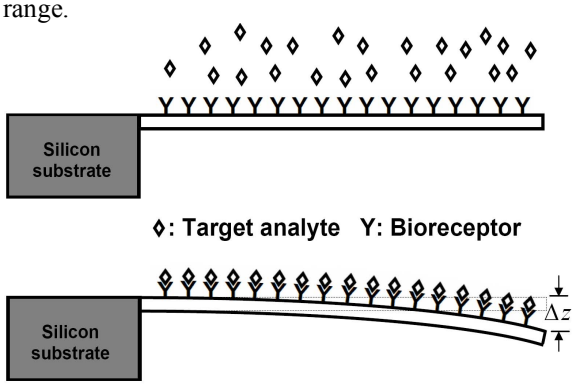

Figure 1. Working principle of a cantilever array biosensor. Cantilever is functionalized by depositing a bioreceptor layer (top); surface-stress induced deflection upon binding between target analyte and bioreceptor (bottom).

With the ability of label-free detection and scalability to allow massive parallelization already realized by cantilever array based biosensors, the next challenge in cantilever biosensor development is achieving a sensitivity in detection range applicable to *in vivo* analysis. The concentrations of some clinically important analytes vary between 10^{-4} to 10^{-15} mol/L. The *in vivo* detection of analytes in such large dynamic range requires an extremely sensitive cantilever. This study proposes a new cantilever design that can assay analytes in extremely low concentrations. This analysis assumes that regardless of underlying mechanism since the deflection produced by either a surface stress or by a concentrated load applied at free end are same, they can be compared. Thus, by comparing the two deflections the surface stress can be equated to the bending stress of cantilever. First, we calculate load by comparing surface-stress induced deflection to its equivalent concentrated load induced deflection. Then this load is applied at cantilever free-end to achieve the original deflection. A finite element analysis software ANSYS is used to analyze the proposed design.

2. Theory

The origin of cantilever motion is not fully understood yet. By using thermodynamic principles in conjunction with DNA hybridization experiments, Wu et al. [5] suggested that cantilever motion is created because of the interplay between changes in configurational entropy and intermolecular energetics induced by specific biomolecular reactions. Further, the entropy contribution can be critical in determining the direction and magnitude of cantilever motion. Hagan et al. [8] reported that the reaction-induced free energy reduction on one cantilever surface is balanced by the strain energy increase due to bending, such that at equilibrium the free energy of the whole system reaches the minimum. Fig.1 shows the working principle of cantilever biosensors. For assaying the target analyte, the cantilever is functionalized by putting a bioreceptor layer onto it. Target analyte is the unknown biomolecules we want to determine. Bioreceptors are analyte-specific molecules. The dimensions of the cantilever sensor, in particular the thickness of the beam, are known to be critical for surface stress sensing applications, as shown by the Stony [9]. For the change in surface stress, σ, defined as the reversible work per unit area required to stretch a pre-existing surface, is related to the deflection of the free end of the cantilever, Δz, is given as [9,10]

$$\Delta z = \frac{4l^2\sigma(1-\upsilon)}{Et^2} \tag{1}$$

where t and l are the thickness and length of the cantilever, respectively; E and υ are Young's modulus and Poisson ratio, respectively.

3. Simulation

The deflections of a rectangular cross-section cantilever beam with fixed-free boundary condition and subjected to a concentrated load at its free end is

$$\Delta z = \frac{4Fl^3}{Ebt^3} \tag{2}$$

where b is width of cantilever and F is applied load. From Eqs.1 and 2, a relation between applied load and the induced surface stress can be given as

$$F = \frac{\sigma bt(1-\upsilon)}{l} \tag{3}$$

Thus the surface-stress induced deflection of the cantilever can be converted into concentrated load induced deflection. In other words, the surface stress induced by the adsorption of biomolecules on the cantilever surface is same as that obtained by applying a concentrated load at cantilever free end. With the surface stress known, Eq. 3 can be used to calculate the concentrated load. Therefore, in simulations instead of using surface stress directly, we applied a concentrated load at cantilever free end to reproduce the adsorption induced surface stresses in the cantilever beam.

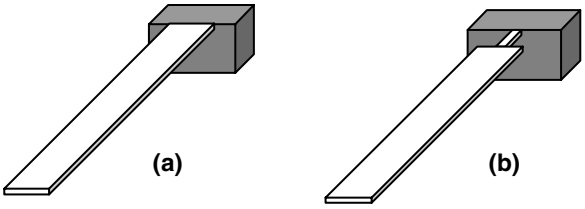

Figure 2. The conventional and proposed cantilever designs; (a) conventional model has uniform cross-section area; (b) proposed model has a narrow throat section near base.

Fig. 2 shows the conventional and proposed cantilever designs. Fig.2(a) shows the conventional design used. Using an array of eight conventional cantilevers, Arntz et al. [1] reported that a maximum surface stress of 0.05 N/m is generated upon injection of 50 µg ml^{-1}(~2.5 µM) myoglobin protein onto the functionalized silicon cantilever, and produced a maximum deflection of 0.9 µm at cantilever free end. The cantilever size was 500×100×50 µm, and its Young's modulus and Poisson ratio was 130 GPA and 0.28, respectively. This experimental data and cantilever model will be used as reference in this study. The surface stress is converted into its equivalent concentrated load using Eq.3. Therefore, the bending induced by a surface stress of 0.05 N/m is equivalent to a bending produced by a concentrated load of 3.6×10^{-9} N applied at cantilever free end. Fig.2 (b) shows the proposed cantilever design. An obvious feature of this design is presence of a narrow strip towards the fixed end. The strip is 50 µm long and 20 µm wide. The lengths and thicknesses of the two models are same. A finite element analysis software ANSYS 11 is used to analyze both cantilever designs. In the analysis of conventional and proposed cantilevers, respectively 125 and 118 4-node SHELL181 elements were used. The computation time on a 2.4 GHz and 1GB memory PC is less than 10s.

4. Results

The simulation result for conventional cantilever design is shown in Fig. 3. For a point load of 3.6×10^{-9} N applied at the free end, a deflection of 0.936 µm is observed. The other end of cantilever is fully constrained. Compared with the cantilever length of 500 µm the deflection amount is negligible, and indistinguishable in the plot. Therefore, for clarity the deflection shown in Fig. 3 is scaled up five times. The deflection magnitude is unchanged.

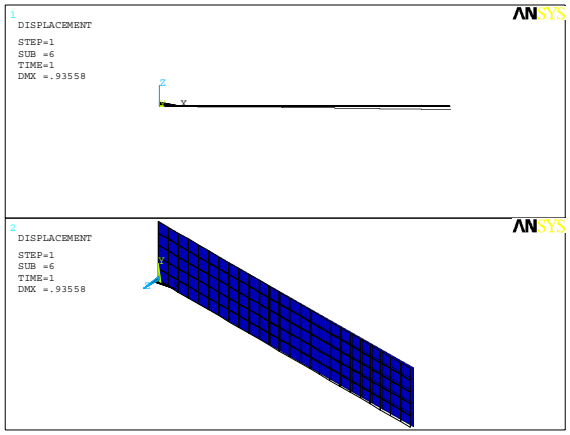

Figure 3. Surface-stress induced deflection of a cantilever beam. Surface stress of 0.05 N/m bends cantilever by 0.936 µm. For clarity, the deflection is scaled up five times.

For a surfaces stress of 0.05 N/m, Arntz et al. [1] reported a maximum deflection of 0.9 µm. The simulation result show similar deflection. For same surfaces stress of 0.05 N/m the simulation result show a maximum deflection of 0.936 µm. The simulation result is about 4% higher than experimental. This apparent discrepancy in deflections can be explained as follows. The difference between experimental and simulation results is due to resistance offered by analyte fluid to cantilever motion. Since the cantilever is submerged in analyte solution, its motion is affected by viscous damping. Therefore, we can conclude that viscosity of analyte fluid reduces the deflection by 4%.

Fig. 4 shows the simulation results of the proposed model. In this case, for the same load of 3.6×10^{-9} N, a deflection of 1.802 µm is observed. This deflection is nearly twice the conventional model deflection. A narrow strip of one-fifth original cross-section area increases the cantilever deflection and hence the sensitivity by nearly two times. Therefore, we can conclude that by introducing a narrow strip toward cantilever fixed end, the flexural stiffness of the

cantilever is decreased significantly, which eventually results in increased deflection. Since compared with the cantilever length the deflection is still very low, the deflection represented in Fig. 4 is also scaled up by five times, as before.

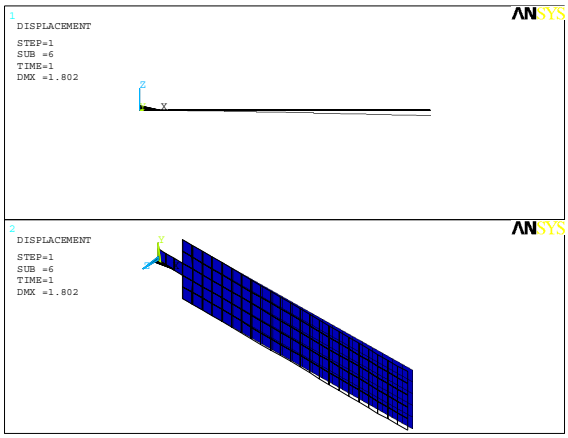

Figure 4. Surface-stress induced deflection of the proposed cantilever beam. Surface stress of 0.05 N/m bends cantilever by 1.802 µm. For clarity, the deflection is scaled up five times.

5. Discussion

From Eq.1 it is clear that for a given surface stress the deflection and hence sensitivity of a cantilever sensor can be increased by either changing the cantilever material or its geometry. Using alternative cantilever material of low Young's modulus and/or low Poisson ratio is one option to achieve higher sensitivity. However, this approach is problematic because in general soft materials have high Poisson ratio. There is one such attempt by Ransley et al. [11]. They demonstrated that a photoresist SU8 could be a potential alternative material to silicon for cantilever biochemical sensing. However, they also reported that SU8 has poor structural integrity and its large thermal expansivity necessitates fine control of the temperature.

Another way to increase the sensitivity, adopted in this study, is changing cantilever geometry. Compared with SU8, silicon is a better structural material with excellent thermal and mechanical integrity. Therefore, almost all of the cantilever array biosensors are made of silicon. It is obvious from Eq.1 that cantilever deflection is directly proportional to the square of length-by-thickness ratio. The length of the cantilever, however, can not be increased much without the loss of miniaturization and parallelization. The cantilever thickness of 0.5 µm cannot be reduced further because it is already too thin and further reduction in thickness would not only affect its structural integrity but also increase its cost. Therefore, to achieve a higher sensitivity without increasing the production cost a modified cantilever design is proposed (Fig. 2(b)).

Fig. 5 shows the conventional and proposed designs of cantilever array biosensors. The conventional model has been used successfully for detection of human proteins [1], DNA hybridizations [5], and human RNA biomarkers [7]. However, the accuracy for proteins and DNA is different and DNA detection is several orders of magnitude more accurate than proteins [1]. Thus, a major challenge of cantilever array design is to overcome limitations such as in detecting proteins and DNA simultaneously using same cantilever array biosensor. The surface stress and deflection behavior of the new cantilever design is shown in Fig.4. It is clear from the figure that for a given surface stress and hence biomolecular interaction, the proposed cantilever design shows more than twice deflection. Since the sensitivity of cantilever depends on its deflection, we can conclude that the new design is more sensitive than the original.

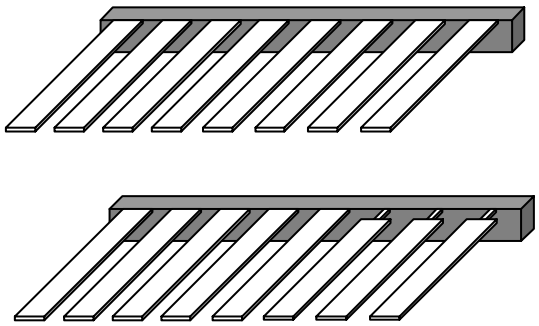

Figure 5. The conventional (top) and proposed (bottom) cantilever array design for biomedical applications.

For *in vivo* detection we need a sensitive biosensor that can assay analytes in large concentration range simultaneously. For such biomedical applications a new array design is proposed (Fig.5). By using an array combination of original and proposed cantilevers on same biochip, a high sensitive biosensor can be designed. Such a sensor can detect simultaneously analytes in extremely large concentration range. The conventional cantilevers in the array can be functionalized with, for example, antigens whose complementary antibodies are found in comparatively higher concentration in the human body. The proposed cantilevers, on the other hand, can be functionalized with bioreceptors whose complementary biomolecules are found in comparatively lower concentrations. Thus,

through proper selection of cantilever types on the array biosensor and proper functionalization, we can design and modify our sensor to the particular requirement. This procedure can be easily adopted to make patient-specific biosensors and immunoassay kits. Such a kit can be used in accurate, simultaneous and real time monitoring of many physiological parameters that indicate a patient's physical condition. For example, if a patient suffers from diabetes there is high probability of infection by secondary diseases such as heart ailments and high blood pressure. So, if we know the person is suffering from diabetes, we can design an immunoassay kit that can not only test the patient's blood insulin level but also some biomarkers proteins such as myoglobin and kinase, whose level can indicate presence of acute myocardial infarction, a type of heart disease.

6. Conclusions

Label-free, rapid, high sensitivity and resolution, and large dynamic range of detection are some of the attractive features exhibited by cantilever array biosensors. These sensors have been successfully used in protein assay, DNA hybridization, and in detection of biomarkers in human RNA. The ultimate goal of microcantilever biosensor design is in *in vivo* biomedical applications where accurate, real time and simultaneous analysis of various clinically important analytes is required. This study proposed and analyzed a new cantilever design which is nearly twice sensitive than conventional design. We showed that surface-stress induced deflection can be equated to concentrated load induced deflection, and a relation between surface and bending stresses can be established. We used this relation to find a load that can induce the same deflection as a surface stress can do. For a given surface stress of 0.05 N/m, which is equivalent to a concentrated load of 3.6×10^{-9} N, the conventional cantilever deflection is 0.936 μm. In contrast, for the same surface stress the cantilevers proposed in this study show nearly twice i.e., 1.802 μm deflection. Therefore, we can conclude that the proposed cantilever design is nearly twice sensitive than the conventional design. Since this study did not consider the viscous resistance by the analyte solution to cantilever motion, the simulation results exceed the experimental results by 4%.

Acknowledgement

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