Piezoelectric Microcantilevers of Nanoscale Thickness for Detection of Cells

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ABSTRACT

The primary goal of the Microfabricated Cell Sensor project is to fabricate microcantilever beams composed of thin films of Lead Zirconium Titanate (PZT), silicon oxide, metal, and a Biotin/Avidin complex. The structures will function as cell sensors based on measurements of the voltage difference produced from the stressed PZT when cells adhere to the cantilevers. The sensor will be used to detect bacterial cells, and in particular, E-Coli. While the process involves fabrication of microcantilevers, it also explores the possibilities of infusing electrically active biological molecules that can aid in inducing electricity in conjunction with the piezoelectric material being used.

Keywords: microcantilevers, piezoelectric, numerical methods, bacteria detection, biotin

1 INTRODUCTION

Microsystems for biological measurements have served as multifunctional, highly sensitive, immunospecific detectors of various types of cells[1]. These sensors can be electrical, optical, mechanical or chemical in nature. Detecting bacteria, virus, and other micro-organisms has become increasingly important over the past several years. In addition to the applications in the medical and food industries, bacteria detection has become important due to terrorist attacks. This project focuses on developing a microsensor to accurately detect bacteria even if they exist in very small quantities.

An initial approach to detect bacteria was centered around a planar array of microelectrodes that operated as oxygen sensors through electrochemical means. This electrochemical microsensor was able to detect aerobic bacteria fairly rapidly, but the minimum quantity of these microorganisms was 10^5 per ml. It was also found that the rate of the oxygen consumption (within the first few minutes) indicated the exact number of bacteria present. Another approach to developing a bacteria measurement device is to design a micromechanical based sensor. This type of device would detect the stress applied to a microcantilever beam via static deflection that can be measured optically or electronically. A device based on this general principle can be very difficult to characterize since quantitative deflections caused by individual cells are not very discrete and an accurate number of cells for such deflections cannot be easily obtained. Therefore, an alternative characterization scheme which allows highly sensitive detection and accurate quantization of individual cells will enhance the functional possibilities and thus result in a more useful sensor.

The approach described in this paper explores the use of a piezoelectric material for highly sensitive detection of cells. The mechanism of piezoelectricity was used because piezoelectric energy conversion produces the highest Maximum Energy Density (MED) during transduction [2]. In addition, piezoelectric materials, being crystalline in structure, provide a geometrically receptive microenvironment for nano-bio-molecular engineering.

2 PROCESSING AND FABRICATION

Design, fabrication and characterization of microcantilevers has been done using numerous materials and techniques by various researchers over the last 15 years [3]. Although several problems in the fabrication of stress-free, micro-environmentally compatible, and deformation resistant cantilevers have been overcome, the processing of these devices has become more complicated by modifications made to simple additive micromachining technology [4]. While manufacturing microcantilevers is central to such a mass-detection project, processing these structures to produce zero stress and maximum compatibility in fluidic environments (including resistance to transient hydrodynamic forces) is not crucial to the production of such exploration devices. This is relatively unimportant because the cantilevers function to discover changes in piezoelectric coefficients that are caused by altering the charge density of piezoelectric material used. Microcantilevers with very low intrinsic stress as compared to no post-processing stress, produced by surface micromachining technology [5] serve as functional detectors for bacteria-sensing. A simple additive surface microfabrication technique (Figure 1) was used for fabrication of the cantilevers.

An RCA cleaned wafer was deposited with a thin sacrificial oxide layer using spin-on glass technology, and etched to produce anchors for the metallic microcantilever structure. In order to realize the beams, sputtering of thin

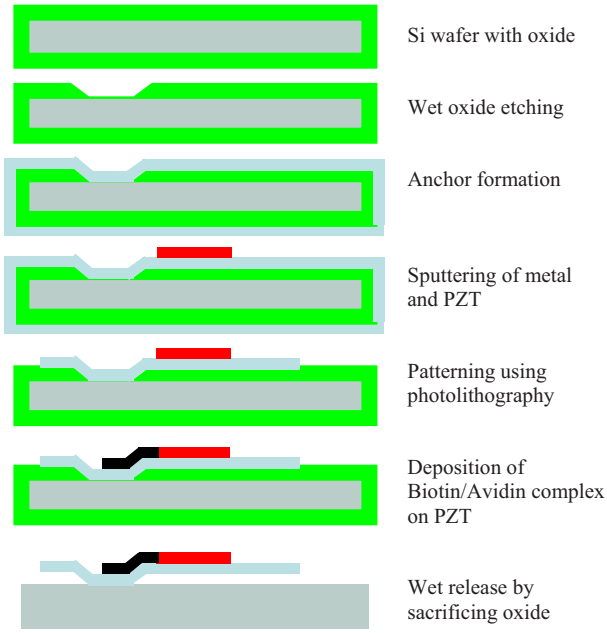

Figure 1: Process flow of fabrication steps

layers of metal of thickness of about 100 nm was carried out. The piezoelectric material, a 99.99% pure PZT target of $Pb(Zr_{0.52}Ti_{0.48})O_3$ stoichiometry and a Zirconate-Titanate ratio of 1.083, was deposited by sputtering. The deposition method of the biotin-avidin complex was intended, but not used in this project and will be presented later, instead a simple dipping technique was chosen. The deposition of metals and PZT was done by a lift-off process. The release of the cantilever beams involves a back-side sacrificial etch of the oxide layer. A numerical simulation of the fabrication process, and the analysis of the electromechanical response of the device was performed, and the results are presented herein.

3 THEORY

3.1 Piezoelectricity-related Theory

The coupling of stress and induced-voltage in a piezoelectric crystal introduces a multidimensional parameter for its characterization, the Coefficient of Piezoelectricity (d), which varies with direction of forces. Initially defined for systems in the macro regime, this parameter has also been characterized for microscaled environments and no particular breakdown of continuum theory has been reported [6]. PZT crystals have a much larger lattice constant (about 25Å), much lower density and fraction of occupation in the lattice, compared to other crystalline materials used in the semiconductor industry. Novel methods for infusion of biological adhesives within polycrystalline structures like PZT are currently being explored. A numerical electrostatic analysis of such a layer is presented.

With an underlying assumption that a biological fluid like Biotin can adsorb and partially diffuse into a low density crystalline material, the properties of a piezoelectric material are expected to be altered by interaction of electrically non-passive foreign biomolecules. In a Biotin/PZT system, piezoelectric constants are altered and/or introduced along an axis parallel to the electric field. While the coupling equation for the process (Equation 1) remained the same as that for the pure PZT case [6],

$$q_i = d^{\varphi}_{ijk}\ D_{ijkl}\ \varepsilon_{lm} + D^{\varphi}_{ij}\ E_j \qquad (1)$$

$$q_i = \sum_{i}^{m} D^{\varphi}_{ij}\ E_j \qquad (2)$$

the piezoelectric constants (Table 1) within the summation term were changed (Equation 2) depending on specific localized cases of the crystal, a function of the fabrication process (among other factors) and varying from experiment to experiment. Here, q_i is the electric "displacement" vector, d^{φ}_{ijk} is the material's piezoelectric strain coefficient matrix, defining the strain ε_{lm} caused by the electrical displacement q_i in an unconstrained material, D_{ijkl} is the materials mechanical elastic constitutive relationship, D^{φ}_{ij} is the materials dielectric property defining the relation between the electrical displacement q_i, and the electrical potential gradient E_j, φ is the electrical potential and E_j is the electrical potential gradient vector, $\partial\varphi/\partial x_i$. Application of additional voltage in this case is not necessary since a frequency shift analysis is not being performed. A coupling equation with zero offset volts still produces induced voltage. Therefore, the permittivity term in equation 1 has been removed for analysis, resulting in equation 2.

3.2 Theory of Mass Detection

Several researchers have previously reported frequency shifts, quantization of deflections produced, and subsequent calculations of detected mass [1,7]. The same principles apply for mass detection with a piezoelectric device, however the process here is more direct. Since the voltage measured can tell us about the quantity of force applied, the measurement of frequency shifts is not needed immediately. By measuring a few micro volts, an accurate measurement of the mass can be made.

3.3 Equations for Electromechanical Analysis

Piezoelectric effect is governed by coupled mechanical equilibrium (Equation 3) and electric flux conservation equations (Equation 4).

$$\int_V \vec{\sigma} \cdot \delta\vec{\varepsilon}\, dV = \int_S \vec{t} \cdot \delta\vec{u}\, dS + \int_V \vec{f} \cdot \delta\vec{u}\, dV \qquad (3)$$

$$\int_V \vec{\sigma} \cdot \delta\vec{E}\, dV = \int_S \vec{t} \cdot \delta\vec{u}\, dS + \int_V \vec{f} \cdot \delta\vec{u}\, dV \qquad (4)$$

where $\boldsymbol{\sigma}$ is the "true" (Cauchy) stress, at a point currently at x, **t** is the traction across a point of the surface, of the body, **f** is the body force per unit volume in the body (such as the d'Alembert force f = -ρ**u**, in which ρ is the density of the body), and $\delta\boldsymbol{\varepsilon}$ is defined as sym $(\partial\delta\boldsymbol{u}/\delta\boldsymbol{x})$, where du is an arbitrary continuous vector field (the virtual velocity field). The electrical flux equation uses **q** as the electric flux vector, q_S as the electric flux per unit area entering the body at a point on its surface; q_V is the electric flux entering the body per unit volume, and $\delta\mathbf{E}$ is defined as $-(\partial\delta\boldsymbol{\varphi}/\delta\boldsymbol{x})$, where $\delta\boldsymbol{\varphi}$ is an arbitrary, continuous, scalar field (the virtual potential).

4 NUMERICAL ANALYSIS

The matrix of piezoelectric constants used to verify the functioning of the device prior to infusion of the Biotin layer is enclosed in the first column of Table 1. The second column has the matrix elements for the Biotin case.

Piezoelectric coefficients matrix	
Without Biotin	**Using Biotin**
$d^{\varphi}_{111} = 0$	$d^{\varphi}_{111} = 0$
$d^{\varphi}_{122} = 0$	$d^{\varphi}_{122} = 0$
$d^{\varphi}_{133} = 0$	$d^{\varphi}_{133} = 0$
$d^{\varphi}_{112} = 0$	$d^{\varphi}_{112} = 0$
$d^{\varphi}_{113} = 0$	$d^{\varphi}_{113} = 0$
$d^{\varphi}_{123} = 0$	$d^{\varphi}_{123} = 0$
$d^{\varphi}_{211} = 0$	$d^{\varphi}_{211} = 0$
$d^{\varphi}_{222} = 0$	$d^{\varphi}_{222} = 0$
$d^{\varphi}_{233} = 0$	$d^{\varphi}_{233} = 0$
$d^{\varphi}_{212} = 0$	$d^{\varphi}_{212} = 0$
$d^{\varphi}_{213} = 0$	$d^{\varphi}_{213} = 0$
$d^{\varphi}_{223} = 0$	$d^{\varphi}_{223} = 0$
$d^{\varphi}_{311} = -.0001$	$d^{\varphi}_{311} = -.0001$
$d^{\varphi}_{322} = -.0001$	$d^{\varphi}_{322} = -.0001$
$d^{\varphi}_{333} = .0003$	$d^{\varphi}_{333} = .0003$
$d^{\varphi}_{312} = 0$	$d^{\varphi}_{312} = .0001$
$d^{\varphi}_{313} = 0$	$d^{\varphi}_{313} = .0001$
$d^{\varphi}_{323} = 0$	$d^{\varphi}_{323} = .0001$

Table 1: Matrix of Piezoelectric Coefficients of PZT used for modeling PZT with & without Biotin-Avidin Complex.

A finite element analysis program was used to solve the equations given in previous sections. The solutions converged to 10^{-6} residual error of convergence. Every run of iterations was performed assuming a certain number of cells in the environment of the detector. Every successive set of iterations increased the number of cells by a factor of 10. A transient computational analysis of the cantilever beam was performed as the cells were forced to impinge on the cantilevers. The force used was proportional to the mass of an E. Coli bacterial cell.

5 RESULTS

5.1 Stress analysis of structure deposited with Biotin-Avidin complex

A deflection of about 18.6 μm was obtained from a beam of length 500 μm and width 100 μm with the Biotin complex, against a static displacement of 17 μm that was obtained by using just PZT, a gain of 9.41% (Figure 2).

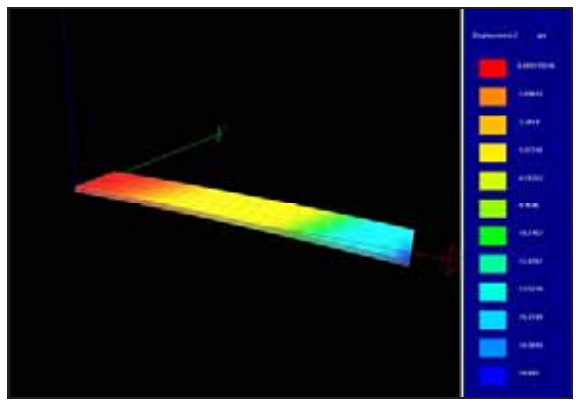

Figure 2: Displacement of the microcantilever beam

5.2 Variation of potential across PZT with Biotin Avidin in a transient simulation

A clear variation of potential across the thickness of the PZT layers was seen for both cases: with and without Biotin (Figure 3). The voltage produced by the transient analysis is shown below (Figure 4) for the application of the mass of one cell.

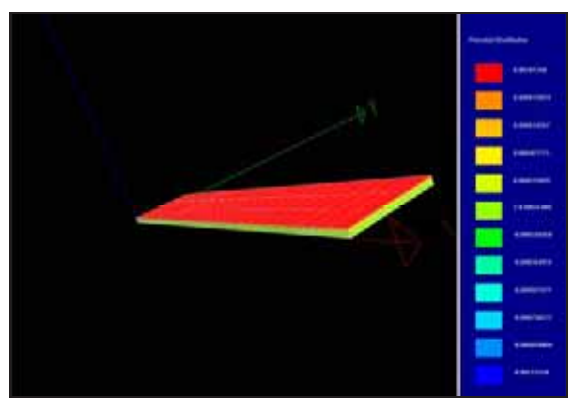

Figure 3: Variation of voltage along the direction of applied stress for the PZT/Biotin complex

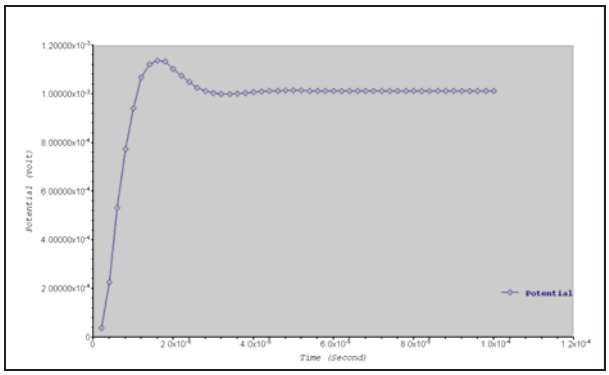

Figure 4: Transient variation of voltage as one bacterial cell impacts the cantilever beam (from the underside)

A more comprehensive comparison of the sensitivity of the microstructure with and without the Biotin/Avidin complex was performed. Figure 5 shows results from simulations of up to 10,000 bacterial cells.

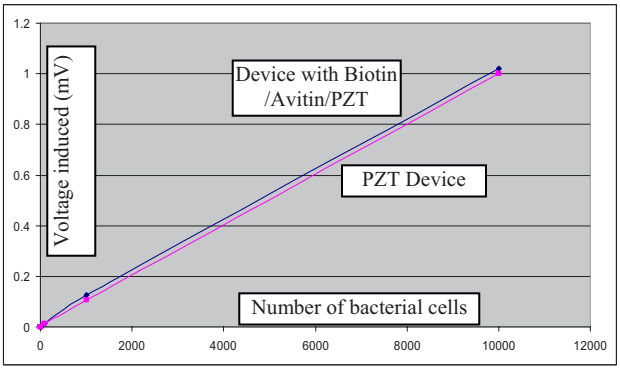

Figure 5: Comparison of sensitivities of the two devices when a very large number of cells are involved

6 SUMMARY AND CONCLUSION

In summary, a theoretical analysis of changes in piezoelectricity that could be induced by disturbance of its crystal structure, was performed. A numerical analysis of the consequences of such an experiment was also performed. It was shown that the Biotin/Avidin complex in a device performs more sensitively than a device that does not use these materials.

7 LIMITATIONS

The most significant disadvantage in employing piezoelectric material for any device is ageing. Loss of piezoelectric properties with time restricts the practical use and operation of a synthetic piezoelectric device, the kind described in this article, to only a few weeks. A minor disadvantage of such a device is operating temperature. While piezoelectric properties have been shown to deteriorate with increase in temperature as approaching Curie temperature, it is unlikely that a detection device will be exposed to extremes in temperature, after fabrication.

8 FUTURE WORK

Obtaining a highly accurate real-time transient response from dynamic input on the cantilever will require monolithic integration of circuitry, but can produce an exact number of adhering cells. It will then be possible to detect cells in very sensitive microenvironments [8].

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