Dominant surface stress driven by biomolecular interactions in the dynamical response of nanomechanical microcantilevers

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Nanomechanical microcantilevers have played a vital role in detecting biomolecular interactions. The ability of microcantilevers to detect biomolecular interactions is ascribed to the principle that the surface stress, caused by biomolecular interactions, dominates the dynamical response of the microcantilever. Here we have experimentally studied the correlation between biomolecular interactions and the dynamical response of microcantilevers. Moreover, the authors employed a mechanical beam model to calculate the surface stress, representing the biomolecular interactions, through measuring the resonant frequency shift. The quantitative analysis of surface stress, driven by the specific protein-protein interactions, demonstrated that microcantilevers enable the quantitative study of biomolecular interactions. © *2006 American Institute of Physics.*
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Nanomechanical microcantilevers have been utilized to gain insight into biomolecular interactions such as protein-protein interactions and protein dynamics.[1–3] It was recently revealed that molecular interactions generate surface stress on a microcantilever surface.[4] This surface stress is usually estimated by using Stoney's equation with measurements from the deflection change induced by biomolecular interactions.[1,5] For microcantilevers operated in the dynamic (oscillating) mode, biomolecular interactions drive the resonant frequency shift.[6–9]

Scaling down of microcantilevers increases the resonant frequency enormously.[10] This improves the sensitivity of microcantilevers for label-free detection of biomolecules because of the much larger frequency shift even for very small amounts of molecular interactions. Accordingly, microcantilevers can be used as immunosensors to monitor specific proteins, which are related to specific diseases, even at a very low concentration in blood serum.

Despite increased interest in oscillating microcantilevers, a quantitative study on the dynamical response change of microcantilevers to biomolecular interactions has not appeared in research publications. In this letter, we report the quantitative analysis regarding the role of surface stress induced by protein-protein interactions on the dynamical response change (i.e., resonant frequency shift) of the microcantilevers. We employed a mechanical beam model in order to understand the relationship between resonant frequency shift and surface stress induced by the protein-protein interaction. Specifically, we consider the dynamical response of microcantilevers driven by the myoglobin antigen-antibody interactions even for low concentrations of antigen ranging from 1 to 100 ng/ml. We also estimate the surface stress on

the oscillating microcantilevers induced by antigen-antibody interactions. The results indicate that the quantitative study of the surface stress can provide insight into biomolecular interactions.

In situ microcantilevers allow the real-time monitoring of antigen-antibody interactions. However, the damping effect of viscous fluid reduces the resonant frequency and consequently degrades the sensitivity of microcantilevers. Here we consider the oscillating microcantilevers in normal air. Let us take into account a cantilever exhibiting the surface stress. The equation of motion for the deflection $w(x,t)$ of microcantilevers is given as

$$\xi \frac{\partial^4 w(x,t)}{\partial x^4} + \frac{\partial}{\partial x}\left[\tau x \frac{\partial w(x,t)}{\partial x}\right] + \mu \frac{\partial^2 w(x,t)}{\partial t^2} = 0, \quad (1)$$

where ξ is the effective flexural rigidity of the microcantilever, τ is the surface stress, and μ is the effective mass per unit length for the microcantilever. For an oscillating microcantilever, the deflection $w(x,t)$ is assumed as $w(x,t) = u(x)\exp[i\omega t]$, where $u(x)$ is the vibration mode and ω is the resonant frequency. Consequently, Eq. (1) becomes the following eigenvalue problem:

$$\xi \frac{d^4 u}{dx^4} + \frac{d}{dx}\left(\tau x \frac{du}{dx}\right) \equiv \wp u = \mu \omega^2 u. \quad (2)$$

The boundary conditions for Eq. (2) are $u(0)=0$, $u'(0)=0$, $u''(L)=0$, and $\xi u'''(L) = \tau L u'(L)$, where L is the microcantilever length. We employ the Ritz method[11] which assumes the deflection mode $u(x)$ in the form of

$$u(x) = \sum_{n=1}^{\infty} \alpha_n \psi_n(x) \equiv \boldsymbol{\alpha} \cdot \boldsymbol{\psi}, \quad (3)$$

where α_n is a constant that should be determined and $\psi_n(x)$ is a basis function. This basis function typically employs a polynomial function that satisfies the essential boundary con-

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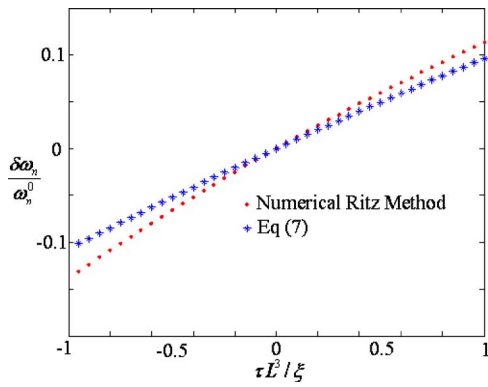[b]Electronic mail: dsyoon@kist.re.kr
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FIG. 1. (Color online) Ritz method allows us to determine the relationship between the resonant frequency shift and the surface stress caused by biomolecular interaction.

FIG. 2. Scanning electron microscopy photograph of the piezoelectric microcantilever array.

ditions, i.e., $\psi_n(0)=0$ and $\psi'_n(0)=0$. Here we use the polynomial basis function $\psi_n(x)$, given as $\psi_n(x)=(x/l)^{n+1}$. From Eqs. (2) and (3), we obtain the numerical linear eigenvalue problem $\mathbf{K}\boldsymbol{\alpha}=\omega^2\mathbf{M}\boldsymbol{\alpha}$ with a stiffness matrix $\mathbf{K}$ and a mass matrix $\mathbf{M}$ defined as

$$\mathbf{K}_{ij} = \int_0^L [\xi\psi''_i(x)\psi''_j(x) + \tau x\psi'_i(x)\psi'_j(x)]dx,$$

$$\mathbf{M}_{ij} = \int_0^L \mu\psi_i(x)\psi_j(x)dx. \tag{4}$$

The numerical linear analysis of the eigenvalue problem provides the resonant frequency as a function of the surface stress,

$$\omega_i = \omega_i^0\left[1 + \frac{2}{\pi^2}\frac{\tau L^3}{\xi}\right]^{1/2}. \tag{5}$$

Here ω_i^0 is the resonant frequency when the microcantilever does not exert any surface stress, i.e., $\omega_i^0=(\lambda_i/L)^2\sqrt{\xi/\mu}$, where λ_i is the parameter satisfying the transcendental equation, i.e., $\cos\lambda_i\cosh\lambda_i+1=0$, and the subscript i indicates the mode i.

For calculation of the surface stress induced by biomolecular interactions, it is assumed that the resonant frequency of a microcantilever without any biomolecular interaction is ω_i^0. After the antigen-antibody interaction on the microcantilever surface, the resonant frequency is shifted to

$$\omega_i \equiv \omega_i^0 + \delta\omega_i = \left(\frac{\lambda_i}{L}\right)^2\sqrt{\left[\frac{\xi}{\mu+\Delta\mu}\right]\left[1 + \frac{2}{\pi^2}\frac{\tau L^3}{\xi}\right]}, \tag{6}$$

where $\Delta\mu$ is the added mass induced by the ligand (antigen) binding. In general, the contribution of added mass by ligand binding to the resonant frequency shift is negligible.[8,12] The surface stress responsible for the resonant frequency shift $\delta\omega_i$ is represented as follows (see Fig. 1):

$$\tau = \frac{\pi^2}{2}\frac{\xi}{L^3}\left[2\left(\frac{\delta\omega_i}{\omega_i^0}\right) + \left(\frac{\delta\omega_i}{\omega_i^0}\right)^2\right] \simeq \pi^2\frac{\xi}{L^3}\left(\frac{\delta\omega_i}{\omega_i^o}\right). \tag{7}$$

Here, in general, for a low concentration of antigen, the ratio $(\delta\omega_i/\omega_i^0)$ is very small. Hence, the quadratic term $(\delta\omega_i/\omega_i^0)^2$ can be neglected in Eq. (7).
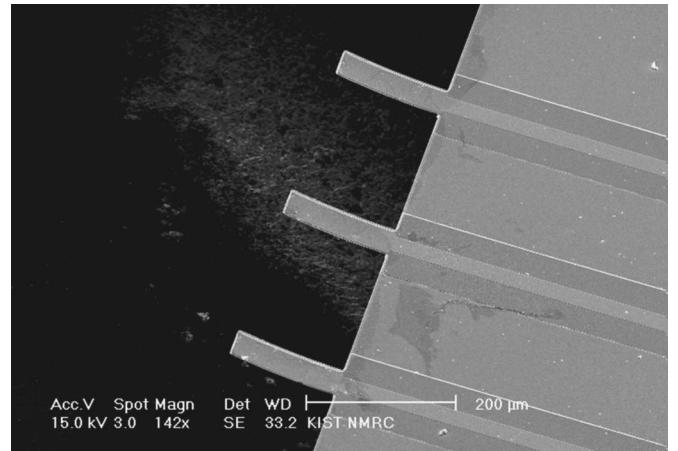
We utilize piezoelectric microcantilevers that are capable of self-actuating/sensing due to piezoelectric and converse-piezoelectric effects. Piezoelectric microcantilevers with dimensions of $150\times50\times2.18$ μm^3 (length×width ×thickness) are fabricated by a micromachining process (for details, review Ref. 7 and see Fig. 2). Antibodies were immobilized on the cantilever surface, which is functionalized with a calixcrown self-assembled monolayer that can bind to ammonium ions of proteins (for details, see Refs. 6 and 13). We used an impedance analyzer to measure the resonant frequency of oscillating microcantilevers actuated by an induced ac signal of 0.5 V p.p. via the electrode of microcantilevers. We measure the resonant frequency of microcantilevers before and after the antigen-antibody interactions. Consequently, the resonant frequency shift, resulting from the antigen-antibody interaction, is evaluated. The microcantilevers are held in a humidity- and temperature-controlled chamber [37 °C, 70% (relative humidity)] during the experiment.

The microcantilever has a length scale on the order of 10^2 μm, and thus the resonant frequency is on the order of 10^2 kHz. Specifically, the resonant frequencies for our microcantilever arrays (six cantilevers for each array) are 74 ± 7 kHz for the first mode and 490 ± 20 kHz for the second mode. This is consistent with the analytical prediction of $\omega_1^0=77.662$ kHz for the first mode and $\omega_2^0=486.739$ kHz for the second mode. The small deviation of experimental data
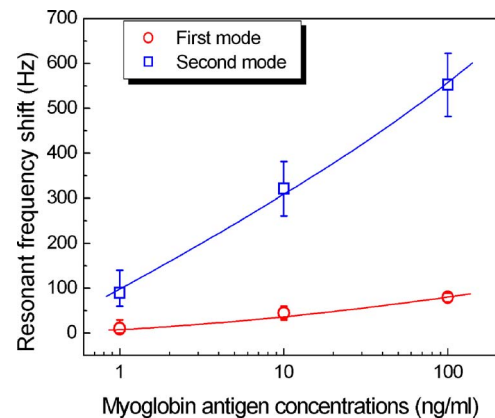

FIG. 3. (Color online) Resonant frequency shift due to a myoglobin antigen-antibody interaction with antigen concentrations ranging from 1 to 100 ng/ml.
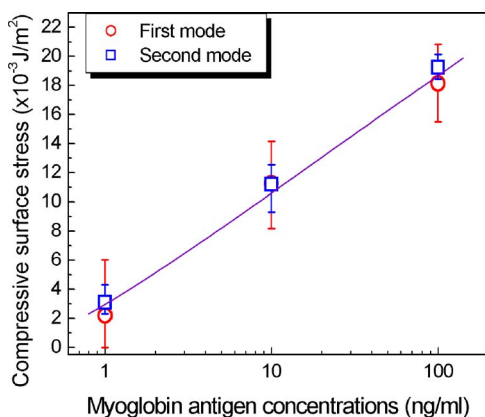
FIG. 4. (Color online) Surface stress exerted on our microcantilevers due to myoglobin antigen-antibody interaction with antigen concentration in a range of 1–100 ng/ml.

from the analytical predictions for the resonant frequencies may be originated from the etching fabrication process. This process might slightly affect the thickness of the cantilevers, resulting in alternation of the resonant frequencies of the microcantilever.

In this work, we consider the resonant frequency shift caused by myoglobin antigen-antibody interactions with the antigen concentrations of 1, 10, and 100 ng/ml. The resonant frequency shift for the first mode induced by the myoglobin antigen-antibody interaction is on the order of 1 Hz for given antigen concentrations. The resonant frequency shift for the second mode is in the range from 100 to 600 Hz for the same antigen concentration (see Fig. 3).

As stated earlier, the resonant frequency shift is originated from surface stress driven by biomolecular interactions. The relationship between the resonant frequency shift and the surface stress due to the biomolecular interactions is provided in the model (see Fig. 1). It shows that the dimensionless resonant frequency shift $\delta\omega_i/\omega_i^0$ is a constant regardless of the mode i, consistent with our results. The dimensionless resonant frequency shift is on the order of -1%, and thus the dimensionless surface stress $\tau L^3/\xi$ is in a range of -10^{-3} to -10^{-2}. Here it should be noted that the negative sign in the resonant frequency shift indicates a decrease in the resonant frequency and that the negative sign for the surface stress represents the compressive surface stress. Despite the small amount of dimensionless surface stress, our microcantilevers are able to recognize the surface stress owing to mechanical properties of our microcantilevers such as a relatively large bending modulus and a small length scale ($\xi = 6.49 \times 10^{-12}$ N m^2, $l = 150$ μm). Specifically, the surface stress exerted on our microcantilevers is in the range of -2×10^{-3} to -2×10^{-2} J/m^2 (see Fig. 4). The role of the mechanical properties of microcantilevers on the surface stress can be understood by considering Ref. 5. In Ref. 5, Arntz *et al.* studied the static deflection behavior of soft microcantilevers driven by myoglobin antigen-antibody interactions with higher antigen concentrations ranging from 20 to 100 μg/ml. In their case, the surface stress evaluated by Stoney's equation is in a range of -2×10^{-3} to -8×10^{-3} J/m^2. This indicates that the soft microcantilevers with larger length scale (e.g., $\xi = 3.02 \times 10^{-13}$ N m^2, $l = 500$ μm for Ref. 5) generate a small amount of surface stress even for large biomolecular interactions. The dimensionless surface stress of the microcantilevers used in Ref. 5

is in a range between 0.82 and 3.79. These values are much larger than the dimensionless surfaces stress of our microcantilevers. This suggests that a cantilever exhibiting a large bending modulus and a small length scale is able to retain a large amount of surface stress in spite of small amounts of biomolecular interactions. Thus, our microcantilevers possessing a large bending modulus and a small length scale allow the very sensitive label-free detection of proteins, even at a very low concentration in solution. Moreover, it is shown that dimensionless surface stress represents the quantity of biomolecular interaction regardless of mechanical properties of the microcantilevers. The surface stress is known to be originated from repulsive intermolecular forces between biomolecules.[2] This is consistent with our result that the antigen concentration representing the packing density of biomolecules (antigen-antibody pairs) is closely associated with the magnitude of surface stress.

In conclusion, we provide the quantitative analysis regarding the role of the surface stress induced by biomolecular interactions on the dynamical response of microcantilevers. It is shown that antigen-antibody interaction induces the compressive surface stress and consequently reduces the resonant frequency. The dimensionless surface stress is the parameter representing the biomolecular interaction regardless of the mechanical properties of the microcantilevers. Hence, the quantitative study of dimensionless surface stress may enable one to gain insight into biomolecular interaction. For future work, the experimental studies on dynamical response of nanoscale (submicron-scale) cantilever to biomolecular interaction will be considered, because the thermal effect (Brownian dynamics) plays a role in dynamical response of nanoscale oscillators.[12,14]

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