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Virus detection using nanoelectromechanical devices

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We have used a resonating mechanical cantilever to detect immunospecific binding of viruses, captured from liquid. As a model virus, we used a nonpathogenic insect baculovirus to test the ability to specifically bind and detect small numbers of virus particles. Arrays of surface micromachined, antibody-coated polycrystalline silicon nanomechanical cantilever beams were used to detect binding from various concentrations of baculoviruses in a buffer solution. Because of their small mass, the 0.5 μm$\times$6 μm cantilevers have mass sensitivities on the order of 10^{-19} g/Hz, enabling the detection of an immobilized AcV1 antibody monolayer corresponding to a mass of about 3×10^{-15} g. With these devices, we can detect the mass of single-virus particles bound to the cantilever. Resonant frequency shift resulting from the adsorbed mass of the virus particles distinguished solutions of virus concentrations varying between 10^5 and 10^7 pfu/ml. Control experiments using buffer solutions without baculovirus showed small amounts ($<$50 attograms) of nonspecific adsorption to the antibody layer. © *2004 American Institute of Physics*.
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High frequency nanoelectromechanical systems[1–6] (NEMS) are being considered as new sensors and devices. They may perform with increased speed and sensitivity in chemical[7,8] and biological[9–12] sensing applications, compared to their macro counterparts. Resonant sensors and actuators must reliably transduce signals and respond repeatably to external chemical and biological environments. The ability to tailor the surface properties with specific biomolecules can be used to create chemically functionalized NEMS oscillators for study of physical chemistry, biocompatibility,[13] DNA hybridization, and biomolecular interactions.[14,15] Recent experiments[16] on the effect of selective molecular binding to the surface of nanomechanical oscillators suggests that it may be possible to detect and study pathogen viral binding by observing their effects on the natural frequency shift of NEMS devices.

In this letter, we have used arrays of chemically functionalized, surface micromachined polycrystalline silicon cantilevers to measure binding events of the baculovirus. Biomolecular binding of the baculovirus to antibody-treated regions of the cantilever sensor alters the total mass of the mechanical oscillator, changing its natural resonant frequency. Due to its high host specificity, baculovirus has been widely used a virus model for biological control. This virus provides a convenient and versatile experimental system used as an efficient eukaryotic gene expression vector. The specific baculovirus used here is *Autographa californica* nuclear polyhedrosis virus (AcNPV), a member of the subgroup A of the family Baculoviridae. The cell lines most frequently used to amplify baculovirus are Sf9 and Sf21, originally established from ovarian tissues of *Spodoptera frugiperda* larvae.

The cantilever fabrication procedure is outlined schematically in Fig. 1. First, a 1-μm-thick sacrificial thermal oxide was grown on the surface of a 10 Ω cm *p*-type Si(100) wafer. Next 150 nm of amorphous Si was deposited using a low pressure chemical vapor deposition (LPCVD) process. The amorphized layer was annealed at 1050 °C for 15 min to alleviate the residual stress resulting in a stress-free polycrystalline silicon film [Fig. 1(a)]. Electron-beam lithography (100 kV Leica VB6) on a polymethyl methacrylate bilayer resist was then used to define the body of the cantilever. A metallic etch mask was generated by evaporating and lifting off a 30-nm-thick chromium layer. Polycrystalline silicon was then etched using reactive ion etching in a CF_4 plasma chemistry [Fig 1(b)]. The sacrificial oxide was removed with hydrofluoric acid, leaving suspended cantilever beams above a Si substrate [Fig. 1(c)]. Figure 1(d) shows an oblique angle scanning electron micrograph of a released cantilever oscillator of length $l=6$ μm, width, $w=0.5$ μm, thickness, $t=150$ nm, with a 1 μm$\times$1 μm paddle.

The device can be modelled as a harmonic oscillator with mass m_{osc} and resonant frequency f_o. Estimated mini-

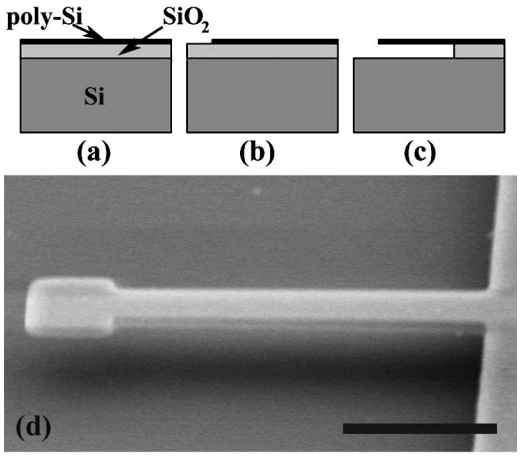

FIG. 1. Fabrication sequence of the nanomechanical oscillators. (a) 1 μm thermal oxidation and LPCVD deposition of the polycrystalline silicon device layer; (b) lithographic definition of the oscillator; (c) sacrificial silicon dioxide is removed in hydrofluoric acid; and (d) scanning electron micrographs of released cantilever oscillators with $l=6$ μm, $w=0.5$ μm, $t=150$ nm with a 1 μm$\times$1 μm paddle. Scale bar corresponds to 2 μm.

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mum detectable surface mass loading Δm is given by

$$\Delta m = k\left[\frac{1}{(f_o - \Delta f)^2} - \frac{1}{f_o^2}\right], \tag{1}$$

where k is the spring constant and Δf is the frequency shift. This equation assumes that the flexural rigidity is unchanged due to subsequent additional mass loading. Assuming $\Delta f \ll f_o$, first-order expansion of Eq. (1) yields

$$\frac{\Delta m}{\Delta f} = \frac{2m_{\text{osc}}}{f_o}. \tag{2}$$

The AcV1 antibody against baculovirus gp64 envelope protein was produced by Bioexpress Cell Culture Service (West Lebanon, NH) with the provided cell line. The purity of the received antibody was confirmed using standard sodium dodecyl sulphate polyacrylamide gel electrophoresis and the binding functionality was characterized using atomic force microscopy. Spodoptera frugiperda (IPLB-Sf21-AE) cells, which were already adapted to EX-CELL 400 serum-free medium (JRH Biosciences, Lenexa, KS), were cultured in an incubator at 28 °C with controlled humidity in order to minimize evaporation. Cell culture initiated with an attached culture at the bottom of a T25 flask with a 5 ml working volume. Suspension of the culture occurred initially in a 50 ml spinner flask and then was transferred to a 250 ml spinner flask (Bellco Glass, Vineland, NJ), with working volumes 40 and 180 ml, respectively. The inoculating cell density was $2-5\times10^5$ cells/ml and agitation speed was 100 rpm. The cells were subcultured every 2–3 days in suspension and every 4–5 days in attached culture until they reached a density of about 2.5×10^6 viable cells/mL. Virus was amplified by infecting exponentially growing Sf21 cells at 1×10^6 cells/mL at a multiplicity of infection of 2. Virus was then harvested for four days post infection. The cells were then centrifuged at 3000 g for 10 min and the supernatant, which contains budded baculovirus, was saved for future use or further purification. Prepared virus solution was titered using BacPAK TM Baculovirus Rapid Titer Kit (BD Bioscience, Palo Alto, CA) by following the protocol provided by the manufacturer. From scanning electron and atomic force micrographs, the baculovirus were approximately 500 nm long and 25 nm in diameter. Inhomogeneity in the length and diameter was about 10% and was directly influenced by the growth stage of the virus.

Following the release, the oscillators were mounted onto a piezoelectric element inside of a vacuum chamber and evacuated to a pressure of 4×10^{-6} Torr. The low operating pressure suppressed viscous damping mechanisms and enabled operation in the regime where the mechanical quality factor was in excess of 10^4. Quality factors of nanomechanical oscillators in air can be as low as 50 when operated in air as biological cell detectors.[10] Signal transduction was achieved by employing an optical interferometric system[3] to measure the frequency spectra resulting from additional loading by the adsorbed mass. The measured out-of-plane oscillations were excited by an external piezoelectric drive mechanism.[16] Baseline spectra [see Fig. 2(a), black curve] were acquired with an optimized driving signal in order to ensure that the device operation was in the linear regime.

Substrates containing oscillator arrays were then immersed into a solution of AcV1 antibodies for 1 h and then washed in de-ionized water and nitrogen dried. The immobilization of the AcV1 antibodies increased the total mass of the oscillators, resulting in reduction of the resonant frequency [see Fig. 2(a) green curve]. Devices were then immersed in a buffer solution with baculovirus concentrations ranging between 10^5 and 10^7 pfu/ml for 1 h. Following the immobilization of baculovirus, devices were rinsed in de-ionized water and nitrogen dried. Devices were then placed into the vacuum chamber and evacuated to a pressure of 4

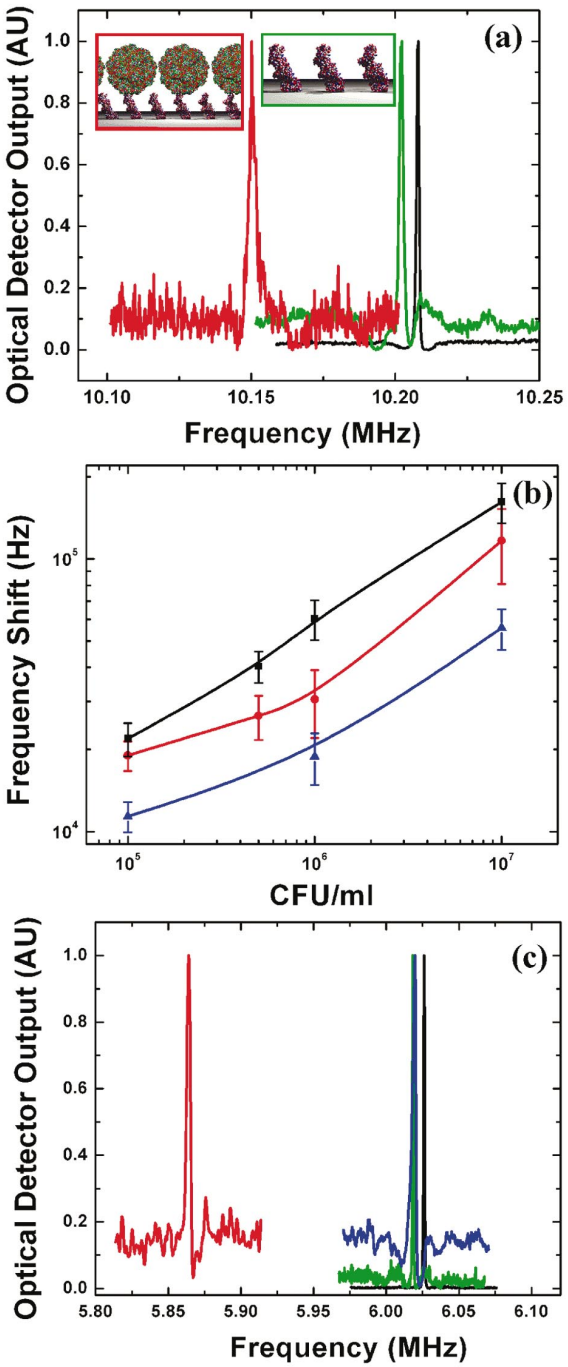

FIG. 2. (Color) Frequency spectra and sensitivity of the nanoelectromechanical cantilevers. (a) Measured frequency spectra of as fabricated $l = 6$ μm oscillator (black), with additional antibody (green) and bacullovirus (red) mass loading. Insets schematically depicts binding of AcV1 to the substrate (left) and immobilization of baculovirus (right) (b) Frequency shift as a function of the baculovirus concentration for 6-μm (black)-, 8-μm (red)-, and 10-μm (blue)-long cantilevers. (c) Frequency spectra of control measurements with $l = 8$ μm cantilevers (black), with antibodies (green), buffer solution without baculovirus (blue) and a buffer solution containing bacullovirus of 10^8 pfu/ml concentration (red).

$\times 10^{-6}$ Torr. The experimental conditions were identical to those during the baseline measurements. The acquired frequency shift was then correlated to the mass of the immobilized baculovirus. Figure 2(a) shows frequency spectra of 6 -μm-long oscillators at various stages of immobilization. From Eq. (2), the minimum detectable masses for the $l=6$, 8, and 10 μm oscillators were 0.41, 0.52, and 0.96 attograms/Hz, respectively. The shift in the natural frequency of the $l=6$ μm oscillator due to the antibody immobilization layer corresponds to a measured mass of 2.29×10^{-15} g. Figure 2(b) shows the frequency shift with the baculovirus concentration for oscillators with $l=6$, 8, and 10 μm. Each data point in Fig. 2(b) represents a collection of measurements from 44 devices. On the lower end of the spectrum, at a concentration of 10^5 pfu/ml, considering a weight of a single baculovirus as $\sim 1.5\times 10^{-15}$ g, we estimate the average number of bound virus particles as 6. These results imply that with a mechanical quality factor of about 10^4, the fabricated oscillators are capable of detecting the binding of a single baculovirus. Our data additionally show that by taking the frequency spectra before and after baculovirus binding, we are able to differentiate various virus concentrations.

Control experiments using a buffer solution without baculovirus were performed to evaluate nonspecific absorption of the buffer solution to the antibody layer [see Fig. 2(c)]. The experimental results from the control experiments show small variations in the frequency shift over many oscillators. In many instances we observed no shift in the frequency. Resulting from 36 devices, the average observed frequency shift during the control measurements was $\sim$126 Hz ($\sim$50 attograms) which signifies a small amount of nonspecific adsorption resulting from the buffer solution. This value is approximately 3.5% percent of the mass of a single virus particle. The amount of nonspecific adsorption can be reduced further by studying the delicate interplay of the chemisorptive antibody interactions with a variety of other buffer solutions. Subsequent exposure of the same set of devices to a buffer solution with a baculovirus concentration of 5×10^8 pfu/ml showed a frequency shift.

Our arrays of resonant nanocantilever detectors distinguished between various solutions of different concentrations of baculovius. These results demonstrate the specificity of the baculovirus binding behavior to the AcV1 antibody. Overall, our data indicate that functionalized NEMS oscillators represent a viable strategy for high sensitivity detection of bound mass. Additionally, since dynamic mode operation of nanoelectromechanical oscillators enables selective detection of virus and antibody binding events, we envision arrays of nanomechanical beams with engineered surface properties, wherein individual elements are functionalized for discriminant biomolecular recognition of a wide variety of pathogens.

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