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3-mercaptopropyltrimethoxysilane as insulating coating and surface for protein immobilization for piezoelectric microcantilever sensors

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We have examined coating $(\text{PbMg}_{1/3}\text{Nb}_{2/3}\text{O}_3)_{0.63}-(\text{PbTiO}_3)_{0.37}$ (PMN-PT)/tin and lead zirconate titanate (PZT)/glass piezoelectric microcantilever sensor (PEMS) with 3-mercaptopropyltrimethoxysilane (MPS) by a simple solution method to electrically insulate the PEMS for in-water applications. In contrast to earlier methyltrimethoxysilane insulation coating, the MPS coating also facilitated receptor immobilization on the sensor surface via bonding of its sulhydryl group to a bifunctional linker, sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate. We showed that a MPS coating of 21 nm in thickness is sufficient to electrically insulate and provide immobilization surface to the PEMS for in-liquid electrical self-excitation and self-sensing. The in-phosphate buffered saline solution resonance spectra were stable with Q values ranging from 41 to 55. The mass detection sensitivities were determined to be 5×10^{-11} and 8×10^{-12} g/Hz for the MPS-insulated PZT-glass and PMN-PT/tin PEMSs, respectively. © 2007 American Institute of Physics. [DOI: 10.1063/1.2727466]

Piezoelectric microcantilever sensor (PEMS) consisting of a highly piezoelectric layer such as lead zirconate titanate¹ (PZT) or lead magnesium niobate-lead titanate^{2,3} $(\text{PbMg}_{1/3}\text{Nb}_{2/3}\text{O}_3)_{0.63}-(\text{PbTiO}_3)_{0.37}$ (PMN-PT) bonded to a nonpiezoelectric layer such as tin is a new type of mass sensor that uses electrical means for detection and can be miniaturized for better mass detection sensitivity.⁴ Most of the electrical insulation coating of miniaturized devices such as piezoelectric/piezoresistive microcantilever sensors and surface acoustic wave (SAW) sensors employed ceramic^{5,6} or polymeric^{7,8} coating deposited by high-vacuum deposition techniques. Other polymeric insulation coatings such as polyimides^{9,10} and benzocyclobutene¹¹ (BCB) can be deposited by wet solution methods, and to be effective they require a thickness of tens of microns, which is too thick for PEMS applications. In a recent study, it was shown that methyltrimethoxysilane (MTMS) about 10 nm in thickness deposited on the tin (the nonpiezoelectric layer side) surface of a PEMS using a sol-gel technique was effective to electrically insulate and allow for complete immersion of the PEMS.¹² However, with this insulation scheme, it was difficult to use the MTMS surface for receptor immobilization. As a compromise, only one electrode of the piezoelectric layer was coated with MTMS for insulation. The other electrode (platinum) surface was coated with a 3-mercaptopropionic acid (MPA) self-assembled monolayer for receptor immobilization. It would be beneficial if an insulation layer can also serve as the linker for receptor immobilization so that

both electrode surfaces can be used for detection. 3-mercaptopropyltrimethoxysilane (MPS) is such a candidate where the silane groups can cross-link to provide the layer thickness¹³⁻¹⁵ and the sulhydryl group can facilitate receptor immobilization.¹⁶ The goal of this study is to show that MPS can not only be a very good insulating coating but also be a surface on which proteins can be immobilized.

Two PEMSs were used in this study. A PZT/glass PEMS was used for the initial testing. The PZT/glass PEMS consisting of a PZT layer (T105-H4E-602, Piezo System, Cambridge, MA) 127 μm thick, 0.7 mm long, 1.4 mm wide bonded to a 150 μm thick glass layer (Fisher Scientific, Pittsburgh, PA) using a nonconductive epoxy (Loctite, Rocky Hill, CT) with a 2.2 mm long glass tip. In the following, we will refer to this PZT/glass PEMS as PEMS-A. Lead magnesium niobate-lead titanate solid solutions/tin (PMN-PT/Sn) PEMS (Ref. 12) was also used. The PMN-PT/Sn PEMS was 560 μm long, 720 μm wide consisting of an 8 μm thick PMN-PT layer bonded to a 6 μm thick tin layer, which we will refer to as PEMS-B in what follows. PEMS-B was constructed first by depositing a 30 nm thick nickel layer with a 15–30 nm thick chromium/nickel bonding layer on one side of the PMN-PT freestanding film by evaporation (e-gun evaporator, Semicore Equipment, Livermore, CA) as the electrode. A 4 μm thick tin layer was then electroplated on the nickel surface at a rate of 500 nm/min as the nonpiezoelectric layer using a plating solution of tin sulfate titrated with sulfuric acid to a $\text{pH}=2.5$. A 150 nm thick platinum was evaporated on the other face of the film as the other electrode. The PMN-PT/Sn bilayer was then embedded in wax and cut to the cantilever shape with a wire saw (Princeton Scientific Precision, Princeton, NJ). After attaching the wires

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to the top and bottom electrodes using conductive glue (XCE 3104XL, Emerson and Cuming Company, Billerica, MA), the PMN-PT/Sn strips were finally glued to a glass substrate to form the microcantilevers.

For the initial MPS deposition, the PEMS was first cleaned in a diluted (1:100 in water) piranha solution [two parts of 98% sulfuric acid (Fisher, Fair Lawn, NJ) with one part of 30% hydrogen peroxide (FisherBiotech, Fair Lawn, NJ)] at 20 °C for 1 min followed by soaking in a 40 mM MPS solution in ethanol covered with paraffin film for 4 h and rinsing by de-ionized (DI) water. They were then soaked in a 0.01M NaOH solution overnight for cross-linking, followed by soaking in DI water for 1 h and overnight vacuum-oven drying (Model 1400E, VWR International) at 762 mm Hg to conclude the first MPS coating. For each of the subsequent MPS depositions, they were soaked in a 40 mM MPS solution in ethanol titrated to $pH=4.5$ with acetic acid and covered with paraffin film for 2 h followed by soaking in DI water for 1 h and overnight vacuum-oven drying at 762 mm Hg. This procedure was repeated two times to give a total of three MPS depositions.

Sulfosuccinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) (Pierce) was used as the bifunctional linker for protein immobilization on MPS. The protein used for this study is an engineered antibody fragment, known as single chain variable fragment¹⁷⁻²³ (scFv) synthesized by Greg Adams at the Fox Chase Cancer Center specific to HER2. HER2 is an epidermal growth factor receptor whose high concentrations have been linked to breast cancer.²⁴ First, the scFv was linked to sulfo-SMCC by mixing 500 μ l of 600 nM scFv solution with 1 ml of 5 mM sulfo-SMCC solution for 1 h for the NHS-ester in the sulfo-SMCC to react with the primary amine of the scFv. Unreacted sulfo-SMCC molecules were then removed by repeating microcentrifugation at 4000 rpm with a 10 kD filter (Millipore) for four times. The MPS-coated PEMS was then soaked in the sulfo-SMCC-linked scFv solution for 1 h to immobilize the scFv on the MPS coating surface via the reaction of the maleimide of the sulfo-SMCC with the sulfhydryl of the MPS.

To investigate the thickness of the MPS coating, we deposited MPS coating on one of the gold electrodes of a 10 MHz quartz crystal microbalance (QCM) (Stanford Research Systems, Sunnyvale, CA) using the procedures described. The initial resonance frequency of the QCM was recorded before MPS coating. After each MPS deposition, the resonance frequency of the QCM was measured. From the resonance frequency shift, Δf_{QCM} , which was the difference of the QCM's resonance frequencies with and without coating, the total coating thickness was then deduced using the following equation,²⁵ $\Delta t = -\Delta f_{QCM} \sqrt{G/\rho} / 2f_{QCM}^2$, where $f_{QCM}=10$ MHz was the natural resonance frequency of the QCM, and $G=2.947 \times 10^{11}$ dyn/cm² and $\rho=2.648$ g/cm³ were the shear modulus and density of the QCM, respectively. The resultant total coating thickness versus number of depositions is shown in Fig. 1 where the term "deposition" is as defined above. The slope as determined by the least squares fit was 7 ± 1 nm/deposition. From the result shown in Fig. 1, the MPS coating on both PEMS-A and PEMS-B, which consisted of three MPS depositions, was estimated to be 21 ± 3 nm.

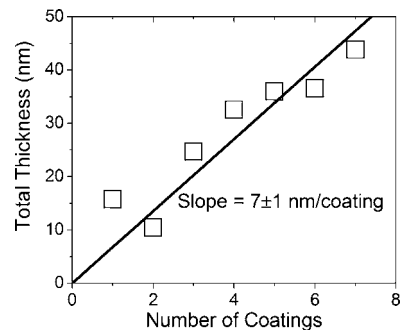


FIG. 1. Total MPS coating thickness vs number of coatings.

To examine the electrical insulation property of the MPS coating, the MPS-coated PEMSs were submerged in a PBS solution. The resultant resonance spectra of the MPS-coated PEMS-A and those of the MPS-coated PEMS-B are shown in Figs. 2(a) and 2(b), respectively, as phase angle versus frequency plots both in air (dashed lines) and in PBS (solid lines). Also shown as inserts in Figs. 2(a) and 2(b) are the optical micrographs of PEMS-A and PEMS-B, respectively. As can be seen both PEMS-A and PEMS-B retained two resonance peaks in PBS, the first and the second flexural peaks in the case of PEMS-A and the second and the third flexural peaks in the PEMS-B case. Note that in Fig. 2(b), the peaks near 120 and 160 kHz are not flexural modes in the longitudinal direction according to our theoretical analysis and therefore are not considered in the present analysis. The

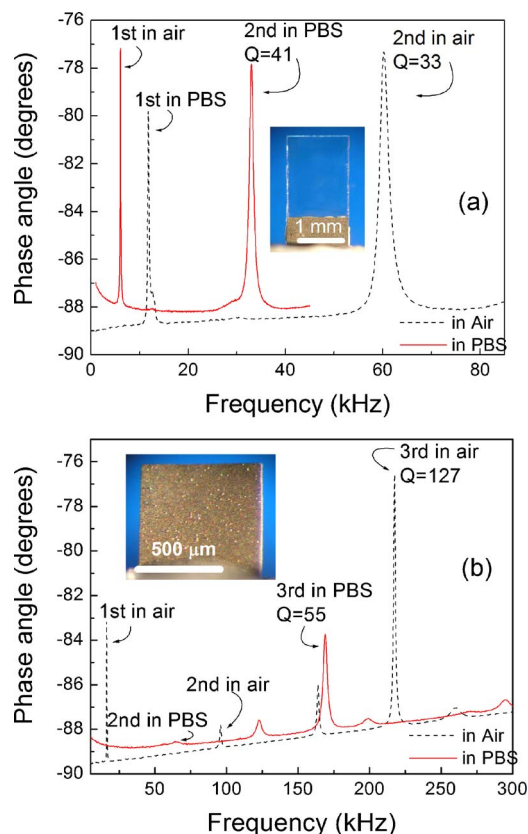


FIG. 2. (Color online) Phase angle vs frequency resonance spectra of (a) PEMS-A and (b) PEMS-B when in air (dashed line) and when submerged in a solution of PBS (solid lines). The inserts in (a) and (b) show the optical micrograph of PEMS-A and that of PEMS-B, respectively.

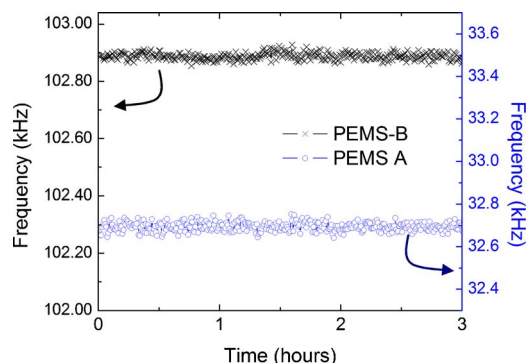


FIG. 3. (Color online) Resonance frequency vs time of PEMS-B (crosses) and PEMS-A (open circles) in PBS. The standard deviations of resonance frequency were 21 Hz for PEMS-A and 13 Hz for PEMS-B, respectively.

reduced resonance peak intensities and resonance frequencies in PBS were, respectively, due to the viscous damping and the mass of the liquid that moved in phase with the PEMS.²⁶ For the PEMS-A the Q value was 33 in air and 41 in PBS, as shown in Fig. 2(a), and for PEMS-B, the Q value was 127 in air and 55 in PBS, as shown in Fig. 2(b). To assess how stable the spectra were in PBS, the resonance peak frequencies of MPS-coated PEMS in PBS were monitored for 3 h. Figure 3 shows the resonance frequency of PEMS-A and PEMS-B versus time over the 3 h period. As can be seen the resonance frequencies of both PEMS remained stable over the time period. PEMS-A displayed a standard deviation of about 21 Hz, and PEMS-B displayed a standard deviation of 13 Hz throughout the 3 h period. These results indicate that the resonance frequencies of the PEMS are stable in PBS solution and can be used to monitor detection in PBS solutions with a background noise not larger than 21 Hz.

To demonstrate the immobilization of protein on MPS coating, we carried out the scFv immobilization procedure on a MPS coated 5 MHz QCM and obtained a resonance frequency shift, $\Delta f_{\text{QCM}} = -40$ Hz, which is reported along with the resonant frequency shift recorded for PEMS-A, PEMS-B, and a control (PBS on MPS-coated QCM) in Fig. 4. The adsorption density, Γ , of the SMCC-linked scFv on the MPS-coated QCM can be estimated using Sauerbrey equation^{25,27,28} $\Delta f_{\text{QCM}} = -(2f_{\text{QCM}}^2 / \sqrt{G\rho})\Gamma$. With

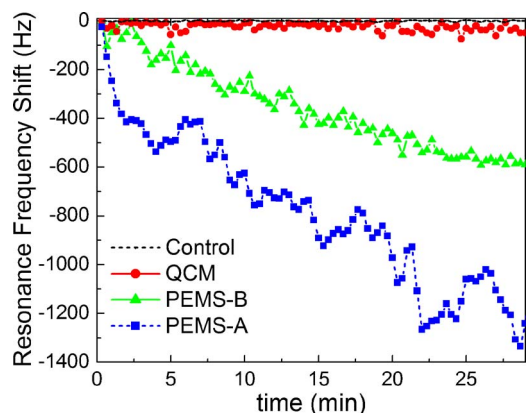


FIG. 4. (Color online) Resonance frequency shift vs time for scFv immobilization using PEMS-A (squares), PEMS-B (triangles), QCM (circles), and a control (PBS on QCM) (dashed line).

$f_{\text{QCM}} = 5$ MHz, and $\Delta f_{\text{QCM}} = -40$ Hz at 30 mins as can be seen from Fig. 4, the Sauerbrey equation gave $\Gamma = 7 \times 10^{-6}$ kg/m². The mass detection sensitivity of the cantilever $(\Delta m / \Delta f)_{\text{cant}}$ can be calculated using the equation $(\Delta m / \Delta f)_{\text{cant}} = \Gamma A_{\text{cant}} / \Delta f_{\text{cant}}$, where Δf_{cant} and A_{cant} were the resonance frequency shift and the areas of the cantilever. Given that the surface area of PEMS-A and that of PEMS-B were 8×10^{-6} and 7×10^{-7} m², respectively, the total masses of the adsorbed SMCC-linked scFv on PEMS-A and PEMS-B were 5.6×10^{-8} and 5.0×10^{-9} g, respectively. With $\Delta f = 1100$ and 600 at $t = 30$ min from Fig. 4, this leads to mass detection sensitivity of $\Delta m / \Delta f = 5 \times 10^{-11}$ and 8×10^{-12} g/Hz for PEMS-A and PEMS-B, respectively.

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