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An investigation of antibody immobilization methods employing organosilanes on planar ZnO surfaces for biosensor applications

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ABSTRACT

One critical aspect for the development of label-free immunosensors is the employment of highly uniform and repeatable antibody immobilization techniques. In this study, we investigated the use of two different silane molecules (3-glycidyloxypropyl)trimethoxysilane (GPS), and (3-mercaptopropyl)trimethoxysilane (MTS) for the immobilization of fluorescently labeled IgG antibodies on planar ZnO surfaces. The chemical modification of the surfaces was investigated using water contact angle measurements, AFM, and fluorescence microscopy. The results of the water contact angle measurements indicate increased surface hydrophobicity after treatment with GPS and MTS as compared to the control. Surface modification was further verified through AFM measurements which demonstrate an increased surface roughness and particle height after treatment with antibodies. The results of the fluorescence studies indicate that the immobilization protocol employing MTS produced 21% higher fluorescence on average with greater uniformity than the GPS-based protocol, which indicates a higher overall density in antibody coverage on the surface of the ZnO. Acoustic sensor tests were employed to confirm the functionality of sensors treated with the MTS protocol. The results indicate that the immobilization protocol imparts sensitivity and specificity to the ZnO-based devices.

1. Introduction

Acoustic wave biosensors function by detecting changes in the path over which the propagating wave travels. These devices are often referred to as gravimetric sensors since the sensing mechanism is related to the addition or subtraction of mass (Ballantine, 1997). As with most types of biosensors, the ability to obtain repeatable results from an acoustic sensor is related to the repeatability of the biolayer. This molecularly specific biolayer typically consist of antibodies, oligonucleotides, or enzymes depending on the sensor application (Chambers et al., 2008). A requirement for high precision biosensors is that the output signal, or response, be repeatable given multiple identical samples and on different sensor elements prepared in the same manner. Therefore controlled, reproducible immobilization of appropriate capture molecules to functionalize the device is necessary to preclude device-to-device signal variations.

The use of zinc oxide (ZnO) has growing interest because it possesses many qualities that make it a good candidate for sensor applications. Primarily, ZnO thin-films can be deposited with excellent *c*-axis orientation using RF magnetron sputtering techniques (Kang et al., 2005) which allows for integration into standard IC fabrication processes. Furthermore, ZnO possesses a relatively strong piezoelectric coupling coefficient of approximately 7–8% and is therefore capable of supporting both longitudinal and thickness shear mode (TSM) waves (Corso et al., 2007), as well as surface acoustic waves (Yamazaki et al., 1980). Additionally, ZnO has become a material of interest because of the ability to grow single crystal ZnO nanostructures with a variety of useful properties (Wang, 2004).

To date, the literature pertaining to functionalizing oxides through covalent methods has largely been focused on quartz (SiO_2). Hydroxyl groups at the surface of an oxide provide sites for reaction with crosslinking molecules to form covalent bonds (Bhatia et al., 1989). One of the more common methods for functionalizing SiO_2 surfaces involves the use of organosilanes. Success with immobilizing antibodies on SiO_2 surfaces has been shown using amine- and thiol-terminal silanes such as 3-mercaptopropyltrimethoxysilane (MTS) with a variety of heterobifunctional crosslinkers including *N*-γ-maleimidobutyryloxy succinimide ester (GMBS) (Bhatia et al., 1989; Shriver-Lake et al., 1997). Heterobifunctional crosslinkers serve the purpose of transforming the end group of the silane into a group that will bind covalently with functional groups on an antibody. Bhatia et al.

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reported that antibody immobilization with a number of different organosilanes yielded a comparable amount of antibodies immobilized from one silane to the next and that the resulting covalently bound biofilm maintained similar overall antigen binding capacity. They also found that there was minimal loss of antibody function after immobilizing the antibodies to the silica surface (Bhatia et al., 1989; Shriver-Lake et al., 1997).

Other work in modifying SiO_2 surfaces has focused on forming self-assembled monolayers (SAMs) using expoxysilanes (Luzinov et al., 2000). One advantage of using an expoysilane such as (3-glycidoxypropyl)trimethoxysilane (GPS) for forming SAMs on oxide surfaces is that it eliminates the need for a secondary crosslinker between the silane molecule and target antibodies. The exposed epoxy groups react readily with amine groups on lysine residues of the antibody. The usefulness of this antibody immobilization technique has been illustrated on indium-tin oxide (ITO) substrates for the development of *Escherichia coli* O157:H7 sensors (Ruan et al., 2002; Yang and Li, 2005).

Despite the amount of research that has been performed on various oxides, including SiO_2, there have been relatively few studies involving surface functionalization of ZnO. One such study reports the use of an amine-terminated silane, 3-aminopropyltriethoxysilane (APS), and glutaraldehyde as the secondary crosslinker to bind a protein, interleukin-6, to the ZnO surface (Krishnamoorthy et al., 2006). One drawback to the use of glutaraldehyde, however, is that it is known to form large polymers which may bind many residues and form multiprotein complexes (Bhatia et al., 1989). Apart from surface treatment with silanes, there have been very few other reports of surface modification studies performed on ZnO. In a study by Sadik et al., adsorption of alkane-thiols to Zn and O terminated ZnO surfaces to form SAMs was investigated, but the study did not involve further functionalization with antibodies or any other type of protein (Sadik et al., 2007). Other studies have described methods for functionalizing ZnO nanostructures, however they are of limited use for translation to planar ZnO surface chemistry because of the morphological and crystalline differences between RF sputtered ZnO surfaces and single crystal nanostructures.

The focus of this work is to investigate antibody immobilization protocols on device-quality sputtered ZnO surfaces. MTS and GPS were deposited onto the ZnO surface using a conventional wet method. Subsequent secondary crosslinking with GMBS was performed for ZnO surfaces coated with MTS. To provide visual confirmation of the density and uniformity of antibody immobilization, fluorescently labeled antibodies were incubated with the surface. The results were investigated using water contact angle measurements, atomic force microscopy, and confocal microscopy. Subsequent sensor tests were performed on ZnO-based resonators functionalized using the MTS + GMBS immobilization method to investigate the functionality of the immobilized antibodies. The results provide a foundation for further research in developing highly uniform antibody immobilization protocols for planar ZnO surfaces.

2. Experimental methods

2.1. ZnO preparation

ZnO samples were prepared by depositing 500 nm of ZnO onto 3″ (1 0 0) silicon wafers (University Wafer) by RF magnetron sputtering using the Unifilm PVD-300 sputtering system. We have previously reported X-ray diffraction (XRD) measurements of the sputtered ZnO films which indicate a strong (0 0 2) ZnO hexagonal 6*mm* crystal orientation (Corso et al., 2007). The wafers were diced into 1 cm × 1 cm squares for surface functionalization experiments. Prior to surface functionalization, the ZnO samples were sonicated in acetone for 5 min in an ultrasonic bath before being cleaned using an ion beam mill (Ion Tech, Inc. MPS-3000). The samples were etched in a 20% oxygen/80% argon atmosphere for 5 min with a beam current of 9 mA at 500 V. This was done to remove any adsorbed chemical species from the surface and to provide a reactive surface for the silane to bind.

2.2. Surface modification

The silane solutions for treating the ZnO samples were prepared under a nitrogen atmosphere: a 4% solution by volume of MTS (Fluka) in dry toluene (Sigma); a 4% solution of GPS (Sigma) in dry toluene. Immediately following the ion milling procedure to clean the ZnO surface, samples were placed in vials containing 2 mL of the silane, sealed, and placed in a nitrogen environment for 24 h. The samples were then removed from the vials and rinsed with ethanol followed by sonication for 5 min in ethanol. The samples were then dried with a stream of N_2. Samples treated with the 4% MTS were then placed in vials containing 2 mL of 2 mM GMBS (Fluka) in ethanol (Sigma) and sealed for 24 h. Following sonication in ethanol, GPS-coated samples were immediately treated with the antibody solution as described below. The MTS + GMBS-coated samples were removed from the GMBS solution and sonicated in ethanol for 5 min. The activated ZnO surfaces were then used for covalent attachment of antibodies. All surface modification steps were performed at room temperature.

2.3. Covalent antibody attachment

The activated surfaces were treated with 546 nm Alexa-Fluor labeled Goat anti-rabbit IgG polyclonal antibodies (Invitrogen) at room temperature. 25 μL of a 200 μg/mL solution of the fluorescently labeled antibodies in PBS buffer (pH 7.4) was pipetted onto the surface of the samples. The antibody solution was incubated for 2 h in an opaque container in a nitrogen environment at room temperature, after which the substrate was rinsed with aliquots of PBS buffer followed by ultra-pure water (Burdick & Jackson) to remove any unbound antibodies from the surface. Control samples for testing simple antibody adsorption were prepared by incubating ion etched ZnO samples with the antibody solution for 2 h.

2.4. Water contact angle measurements

Water contact angle measurements were performed using an SEO Phoenix 150 Contact Angle Analyzer. The contact angles for each of the surface treatments were evaluated by averaging the contact angles from at least five separate measurements. The silanized samples were measured and compared to a control sample of untreated, ion etched ZnO. The measurements were used as a semi-quantitative method of determining the film quality and reproducibility as well as to confirm that the surface was being chemically modified. ANOVA was used to analyze the water contact angles for each of the surface treatments.

2.5. AFM measurements

A Digital Instruments (DI) 3000 AFM was used in tapping mode to measure the following: bare ZnO samples; samples after treatment with MTS + GMBS and GPS, respectively; samples after treatment with the croslinkers + antibody solution. AFM probe tips were obtained from Veeco Instruments (FESP). Multiple scans of each sample were taken to across each 1 cm × 1 cm sample and scans were taken on multiple samples. The scans were evaluated for

surface roughness and overall average particle height and diameter using the DI Nanoscope software.

2.6. Confocal microscopy

Following the antibody conjugation reaction, the devices were dried with a stream of N_2 and fixed to a glass slide in preparation for confocal microscopy analysis. A Zeiss Laser Scanning Microscope (LSM) 510 equipped with a HeNe laser (543 nm excitation) was used to visualize the distribution and intensity of the fluorescently labeled antibodies. Each sample was viewed at 10× and 40× magnification and images were taken at random locations throughout the area of antibody deposition. Control samples were prepared and analyzed for comparison between fluorescence due to antibody coverage and fluorescence to due background noise. Rather than incubation with the 200 μg/mL antibody in PBS, the control samples were treated with the full crosslinking protocol then incubated with 25 μL of 5 mM azide in PBS to replicate the solution containing the Alexa-Fluor labeled antibodies. Therefore, the only difference between the control and target samples is the presence of antibodies. To quantify the fluorescence for each surface treatment, the detector gain was fixed at a constant value and images were taken for all of the samples. The RGB luminance intensity signal was collected for each pixel in 0.03 mm^2 areas across the samples. The average fluorescence intensity was calculated by taking the mean pixel luminance intensity for all pixels within a 0.03 mm^2 area over at least 25 separate measurements. Statistical analysis was performed using ANOVA to compare the average fluorescence intensity of each surface treatment.

2.7. Sensor testing

In order to assess whether the antibodies remain functional after the immobilization procedure, sensor tests were performed using the MTS silanization protocol to functionalize ZnO thickness shear mode acoustic resonators. These devices are excited with a lateral field excitation electrode configuration, which removes the electrodes from the active area of the device (Corso et al., 2007; Rosenbaum, 1988). The absence of electrodes in the active region of the device necessitates a surface treatment protocol capable of linking the antibodies directly to the ZnO surface. The ZnO TSM resonators employed here are sensitive to perturbations such as mass loading events which produce a shift in the resonant frequency of the device. Here, we test the functionality of the antibody immobilization procedure by exposing the functionalized sensors to solutions containing mesothelin—a protein that is highly expressed in several cancers including mesotheliomas, ovarian and pancreatic cancers, and some squamous cell carcinomas (Argani et al., 2001; Chang and Pastan, 1996; Chang et al., 1992; Ordonez, 2003).

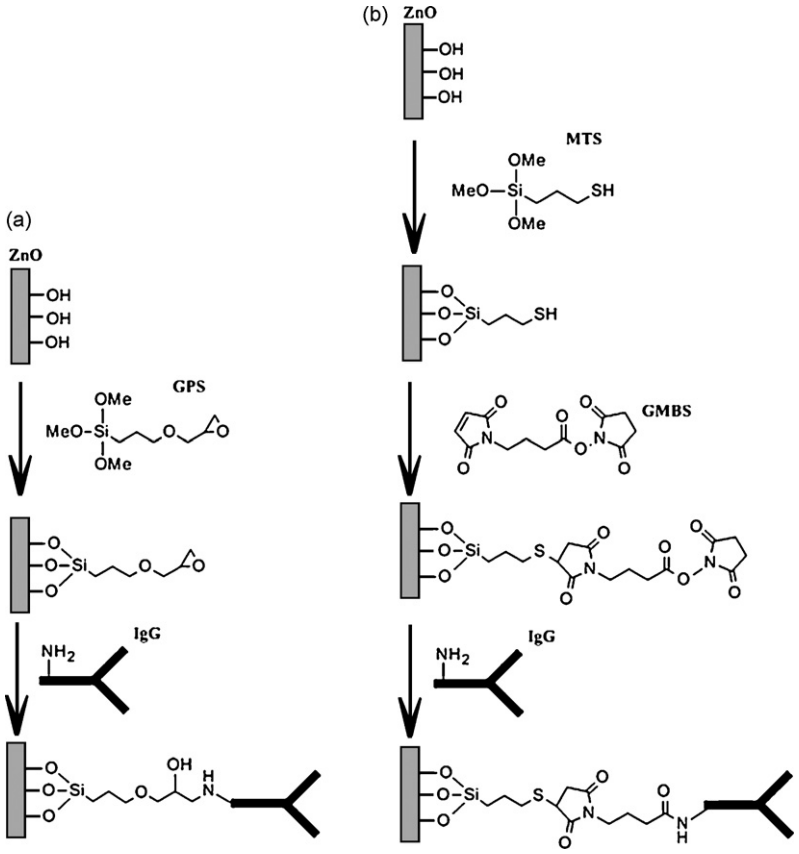
The acoustic sensor devices were fabricated as previously described (Corso et al., 2007). An acoustic mirror of 8 alternating layers of 640 nm of W and 1000 nm of SiO_2 were deposited on a Si wafer. ZnO was RF-sputtered to a thickness of 750 nm to produce devices that have a fundamental operating frequency at approximately 2 GHz. Three 200 nm long electrodes were patterned on the surface of the ZnO with a gap of 30 nm between them for generating and maintaining the acoustic resonance. Functionalization of the resonators was performed using the MTS silanization protocol as described above. Antibody solutions were incubated with the surfaces for 2–3 h and were subsequently washed with buffer followed by purified H_2O. For the mesothelin tests, the target sensors were coated with 20 μg/mL of the MB IgG_{2a} mouse monoclonal antibody solution. The mAb MB antibodies were developed in the laboratory of Ira Pastan at the National Cancer institute. They have been shown to detect mesothelin by immunohistochemistry, fluorescence-activated cell sorting, and ELISA and have high affinity for the mesothelin protein (Onda et al., 2005). The reference sensors were coated with a 20 μg/mL solution of anti-FITC monoclonal IgG antibodies (sc-69871—Santa Cruz) because fluorescein isothiocyanate (FITC) is a synthetic molecule that is not present in the samples of interest.

A series of tests were performed to independently analyze the sensor response to buffer solutions spiked with two different concentrations of purified mesothelin-rFc. Mesothelin-rFc is a recombinant fusion protein which was also developed in the laboratory of Dr. Ira Pastan at the NCI which consists of the 40 kDa COOH-terminal mesothelin protein fused to rabbit Fc (Onda et al., 2005). The mesothelin-rFc fusion protein has been used for developing ELISA calibration standard curves in the past and contains the entire extracellular portion of mesothelin (Hassan et al., 2006).

Following functionalization of the acoustic sensors, the devices were placed in shallow dishes of pH 7.4 PBS buffer to keep the antibody coating hydrated for subsequent antigen binding. The sensors were then tested using an HP8753 network analyzer to record the impedance response of the devices and to establish the initial resonance frequency of each device. The sensors were then incubated with 25 μL of the mesothelin-rFc solution for 25 min. Sensor tests were performed on solutions of mesothelin-rFc in PBS buffer with concentrations of 10 ng/mL, and 10 μg/mL. After the incubation period, the sensors were rinsed with buffer and water to remove any unbound protein, and were then re-tested using the network analyzer to record the post-exposure impedance response. Sensor responses from before and after exposure to the sample were analyzed. The frequency shifts represent the different between the perturbed and unperturbed resonance frequency. Average frequency shifts across multiple sensors for each solution concentration were calculated and plotted. One-way ANOVA was used to evaluate the statistical significance between the frequency shifts and the concentration of sample solution.

3. Results and discussion

Based on previously described mechanisms (Bhatia et al., 1989; Krishnamoorthy et al., 2006; Yang and Li, 2005), a summary of the reactions for covalently attaching antibodies to the ZnO surface is shown in Scheme 1. Deposition of the silanes is achieved through reaction of the head groups with hydroxyl groups at the ZnO surface. The thiol group on the MTS molecules react with the maleimide region of the secondary crosslinker GMBS in organic solvent. This reaction leaves the succinimide residue of the GMBS available for antibody attachment. The succinimide residue then binds to an available amino group of the antibody to form a covalent bond between the antibody and the ZnO surface. It is important to note, however, that targeting amine-groups on the antibodies does not promote any specific orientation of the antibodies since amine-containing residues such as lysine can be located on many parts of the antibody. Despite this, it has been shown that antibodies immobilized using this approach still maintain a significant amount of activity, albeit less than with oriented immobilization techniques such as those that target the carbohydrate group on the Fc region of the IgG antibody (Shriver-Lake et al., 1997). Additionally, the ease and simplicity of the immobilization protocols studied here make them an attractive option for biosensor applications over oriented immobilization techniques which often require numerous steps, harsh experimental conditions, and high antibody losses prior to immobilization.



Scheme 1. The immobilization scheme for covalent attachment of IgG antibodies to the ZnO surface using (a) GPS and (b) MTS followed by the secondary crosslinker GMBS.

3.1. Water contact angle measurements

The results from the water contact angle (WCA) measurements after the samples were coated with the silanes indicate that the average WCA for each of the crosslinkers were greater than the WCA of the control, indicating an increase in surface hydrophobicity. Samples coated with MTS had an average water contact angle of $77^\circ \pm 1$ which were statistically different than the untreated ZnO (control) case which exhibited a water contact angle of $72^\circ \pm 1$ ($p = 0.002$). The contact angle results for surfaces coated with MTS seem to agree well with previous WCA investigations of thiol-terminated monolayers on gold which were reported to be approximately 83.5° on average (Tlili et al., 2007). The WCA measurements performed with the 4% GPS solutions also suggest that the surface is being chemically modified. The samples coated with GPS yielded an increase in average WCA of $80^\circ \pm 5$ immediately after the water droplet is placed onto the sample surface. Over the ensuing 20–30 s the water contact angle drops by about 5–10° and then stabilizes. One possible explanation for this phenomenon is that the water droplets react with the exposed epoxy groups, hydrolyzing the ring to form surface hydroxyl groups which would result in a more hydrophilic surface. These WCA results are relatively higher than those reported for MTS and GPS on SiO_2 which were found to be 58° and 52° on average, respectively (Bhatia et al., 1989).

3.2. AFM measurements

AFM was used for monitoring the morphological changes at the surface that result from coating the surface with the crosslinkers and subsequent immobilization of antibodies. Fig. 1 shows stepwise AFM scans for each surface after treatment with the crosslinkers and antibody solution. Fig. 1(a) shows the characteristic cobblestone appearance of the surface of the untreated control ZnO sample, with round grains that have fairly well defined boundaries. For the bare ZnO case, the mean surface roughness is approximately 2.8 (±0.4) nm while the particle height is 3.05 (±0.5) nm. The characteristic AFM scan of the surface after silanization with GPS in Fig. 1(b) shows a surface that is morphologically different from that of the controlled case. The average surface roughness decreases on average by 57% to 1.2 (±0.3) nm. This implies an overall smoothening of the surface which is similarly suggested by the appearance of the scan. One likely explanation is that the GPS molecules have been deposited into the gaps in-between the grains to fill some of the grain boundaries. The results were observed at many locations across any single chip, and from chip to chip. After incubation with the antibody solution, the GPS + Ab treated surface has an increased surface roughness on average to 2.0 (±0.1) nm with an average particle height of 3.3 (±0.8) nm which indicates that the surface is being modified after treatment with the antibody solution. A typical scan is shown in Fig. 1(c).

The AFM scans of the samples treated with MTS + GMBS in Fig. 1(d) show significant differences in the surface morphology as compared to the GPS. Rather than a decrease in surface roughness, we see an increase in surface roughness to 3.7 (±0.7) after treatment with MTS + GMBS. The average particle height for the MTS + GMBS treated samples was increased to 6.8 (±0.4) nm indicating an increase in particle size due to deposition of the MTS + GMBS. Fig. 1(e) clearly shows the subsequent treatment with the antibody solution yields particles on the surface that have a larger average height 13.7 (±0.3) nm which is indicative of the immobilization of the antibodies to the surface. The data from these scans along with the WCA measurements provide confidence that the surface is being chemically modified and that the surfaces are

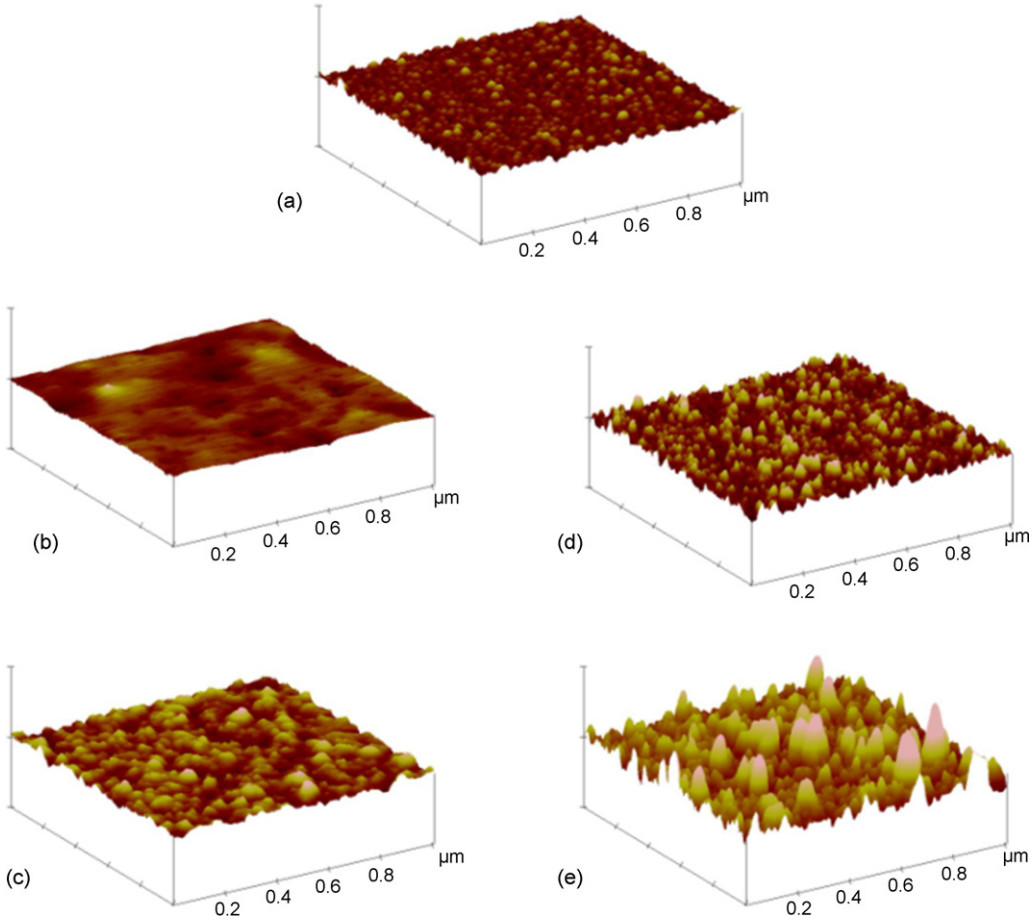


Fig. 1. AFM topographical scans of (a) the untreated ZnO surface and samples treated with (b) GPS, (c) GPS + Ab, (d) MTS + GMBS, and (e) MTS + GMBS + Ab. The scan sizes are 1 μm × 1 μm with a vertical scale of 65.0 nm.

morphologically modified after treatment with the antibody solution.

3.3. Fluorescence microscopy measurements

While the AFM scans tend to indicate that the surfaces are being modified with the surface treatment, fluorescence microscopy was employed to provide more information about antibody surface coverage density and uniformity. Prior studies characterizing antibody immobilization techniques have generated a variety of methods for analyzing the amount of antibodies bound to the surface of a material (Bhatia et al., 1989; Shriver-Lake et al., 1997; Yang and Li, 2005). As acoustic biosensors become increasingly more compact, the antibody immobilization surface coverage becomes increasingly more important. It is necessary to compare the surface treatments quantitatively, which is demonstrated in this study through an analysis of the fluorescence. Fig. 2 shows a comparison of the average fluorescence intensity (luminescence) for the two silane immobilization methods vs. the untreated bare ZnO sample. Included are the average fluorescent intensities for the control samples which serve as an estimation of the combined background fluorescence from the ZnO, the crosslinkers (for the GPS and MTS treated chips), and the PBS buffer. The average intensity was found to be highest for the MTS as compared to the GPS and the ZnO. The fluorescence of the bare ZnO sample treated with antibodies above the control can be attributed to a small amount of antibody adsorption to the surface of the ZnO in the absence of crosslinking molecules. The fluorescence of the samples treated with the MTS were on average about 41% higher than the samples treated with GPS ($p < 0.001$). Since level of the fluorescence within a region of constant size is related to the amount of fluorophor present, these results indicate increased antibody surface coverage with the MTS over the GPS. The MTS fluorescence as compared to the untreated ZnO case indicates roughly a three-fold increase of average fluorescent intensity ($p < 0.001$). The uniformity of the antibody coverage can be correlated to the standard deviation in pixel luminance for each of the

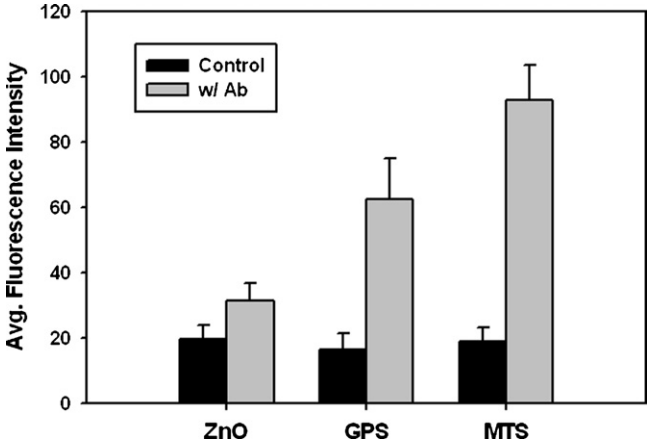


Fig. 2. The average fluorescence intensity for each surface treatment after incubation with antibodies vs. incubation with buffer. A significant difference between MTS and GPS was calculated with ANOVA to be $p < 0.001$. (Error bars = standard deviation).

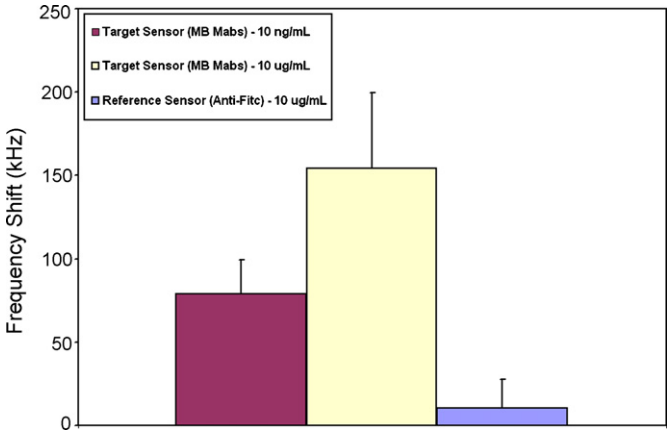


Fig. 3. Average frequency shift measured in the target and reference ZnO-based acoustic sensors after exposure to mesothelin-rFc solutions. $n = 6$ for each test. (Error bars = standard deviation).

samples. We found a lower standard deviation for surfaces treated with MTS (12.2 a.u.; 12.6% of the average) as compared to GPS (18.3 a.u.; 26.4% of the average) which indicates an overall higher uniformity in over 25 separate measurements across multiple samples which include ~9500 pixels each. The increased fluorescence observed in samples treated with MTS over those treated with GPS was repeatable from sample to sample which also indicates robustness in the protocol with each of these surface treatments.

3.4. Sensor functionality testing

Based on results from the fluorescence studies, it was concluded that the high uniformity of the MTS immobilization protocol made it more suitable for sensor detection studies than GPS. However, fluorescence studies alone can only indicate where the antibodies are located, but give no information about whether or not the antibodies are functional. To test this, TSM acoustic sensors were employed (Fig. 3).

The sensor tests were performed with two concentrations of purified mesothelin-rFc solutions which resulted in increasing shifts, on average, for the target sensors over the reference sensors. The MB-coated sensors tested with a concentration of 10 ng/mL ($n = 6$) had an average frequency shift of 78 kHz. In contrast, the average frequency shift for MB-coated sensors exposed to a 10 μg/mL solution ($n = 6$) was found to be 154 kHz. This increased frequency shift observed with an increased concentration of the target marker was found to be statistically significant ($p = 0.004$). The reference sensors were tested with a concentration of 10 μg/mL and had an average frequency shift of 10.6 kHz which is significantly lower than the average shifts measured in the two sets of tests performed with sensors coated with MB antibodies ($p < 0.001$). The difference in the response of the target sensors to the two samples of varying concentration is indicative of the activity of the antibody coating after the immobilization protocol. Furthermore, the fact that the target responses are significantly greater in magnitude than those of the reference sensors confirms that the antibody coating imparts molecular specificity to the sensors.

Since the FITC antigen is not present in the samples that were tested, the shift observed in the reference sensors coated with anti-FITC antibodies can be attributed to non-specific protein adsorption to the sensor surface. The issue of non-specific protein adsorption must be considered in any label-free sensor system. With acoustic sensor platforms, the use of reference sensors is a common method for mitigating the effects on non-specific protein adsorption on sensor detection outcomes (Corso et al., 2006; Lee et al., 2003). This is because the reference sensor response can be subtracted from the target sensor response to give a signal due to specific antibody–antigen interactions only. However, for sensor systems where a reference sensor is not feasible or not desired, other methods can be implemented to block non-specific protein adsorption such as treating the surface with bovine serum albumin (BSA) prior to exposure to the target sample (Bhatia et al., 1989).

4. Conclusions

We have provided an investigation of two antibody immobilization techniques on planar ZnO surfaces. The surfaces were analyzed using water contact angle measurements, atomic force microscopy, and fluorescence microscopy to determine the surface characteristics and antibody surface coverage. We found that protocols employing both GPS and MTS are effective in immobilizing antibodies to the ZnO surface, but that MTS offers greater surface coverage, on average. We achieved uniform and repeatable surface coverage using both methods which is a necessary requirement for biosensor functionalization. The antibody functionality was investigated using ZnO-based acoustic resonators. Target sensors treated with the MTS protocol were found to be sensitive to solutions containing mesothelin-rFc and an increased frequency shift was observed corresponding to exposure to solutions with a higher concentration of the protein.

This study is offered as an initial study for immobilization of antibodies on planar ZnO surfaces. Functionalization of crystalline ZnO surfaces is becoming an increasingly important topic as new biosensor platforms are being developed with this material. Further work should build upon the studies detailed here for optimizing immobilization times to maximize the density and uniformity of covalent antibody immobilization procedures. It is concluded that MTS is a viable option for moving forward in functionalizing ZnO-based sensors due to the fact that it is a commercially available chemical and provides high antibody surface coverage with good uniformity.

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References

Argani, P., Iacobuzio-Donahue, C., Ryu, B., Rosty, C., Goggins, M., Wilentz, R.E., Murugesan, S.R., Leach, S.D., Jaffee, E., Yeo, C.J., Cameron, J.L., Kern, S.E., Hruban, R.H., 2001. Clin. Cancer Res. 7 (12), 3862–3868.
Ballantine, D.S., 1997. Acoustic Wave Sensors: Theory, Design, and Physico-Chemical Applications. Academic Press, San Diego.
Bhatia, S.K., Shriver-Lake, L.C., Prior, K.J., Georger, J.H., Calvert, J.M., Bredehorst, R., Ligler, F.S., 1989. Anal. Biochem. 178 (2), 408–413.
Chambers, J.P., Arulanandam, B.P., Matta, L.L., Weis, A., Valdes, J.J., 2008. Curr. Issues Mol. Biol. 10, 1–12.
Chang, K., Pastan, I., 1996. Proc. Natl. Acad. Sci. U.S.A. 93 (1), 136–140.
Chang, K., Pastan, I., Willingham, M.C., 1992. Int. J. Cancer 51 (4), 548–554.
Corso, C.D., Dickherber, A., Hunt, W.D., 2007. J. Appl. Phys. 101 (5), 54511–54514.
Corso, C.D., Stubbs, D.D., Lee, S.H., Goggins, M., Hruban, R.H., Hunt, W.D., 2006. Cancer Detect. Prev. 30 (2), 180–187.
Hassan, R., Remaley, A.T., Sampson, M.L., Zhang, J., Cox, D.D., Pingpank, J., Alexander, R., Willingham, M., Pastan, I., Onda, M., 2006. Clin. Cancer Res. 12 (2), 447–453.
Kang, D.J., Kim, J.S., Jeong, S.W., Roh, Y., Jeong, S.H., Boo, J.H., 2005. Thin Solid Films 475 (1–2), 160–165.

Krishnamoorthy, S., Bei, T., Zoumakis, E., Chrousos, G.P., Iliadis, A.A., 2006. Biosens. Bioelectron. 22 (5), 707–714.
Lee, S.H., Stubbs, D.D., Cairney, J., Hunt, W.D., 2003. Proceedings of IEEE Sensors 2003, Toronto, Ont., Canada, pp. 1194–1198.
Luzinov, I., Julthongpiput, D., Liebmann-Vinson, A., Cregger, T., Foster, M.D., Tsukruk, V.V., 2000. Langmuir 16 (2), 504–516.
Onda, M., Willingham, M., Nagata, S., Bera, T.K., Beers, R., Ho, M., Hassan, R., Kreitman, R.J., Pastan, I., 2005. Clin. Cancer Res. 11 (16), 5840–5846.
Ordonez, N.G., 2003. Am. J. Surg. Pathol. 27 (11), 1418–1428.
Rosenbaum, J.F., 1988. Bulk Acoustic Wave Theory and Devices. Artech House, Boston.
Ruan, C.M., Yang, L.J., Li, Y.B., 2002. Anal. Chem. 74 (18), 4814–4820.
Sadik, P.W., Pearton, S.J., Norton, D.P., Lambers, E., Ren, F., 2007. J. Appl. Phys. 101 (10), 104514–104515.
Shriver-Lake, L.C., Donner, B., Edelstein, R., Breslin, K., Bhatia, S.K., Ligler, F.S., 1997. Biosens. Bioelectron. 12 (11), 1101–1106.
Tlili, A., Abdelghani, A., Aguir, K., Gillet, M., Jaffrezic-Renault, N., 2007. Mater. Sci. Eng. C-Biomimetic Supramol. Systs. 27 (4), 620–624.
Wang, Z.L., 2004. Annu. Rev. Phys. Chem. 55, 159–196.
Yamazaki, O., Mitsuyu, T., Wasa, K., 1980. IEEE Trans. Sonics Ultrasonics SU-27 (6), 369–379.
Yang, L., Li, Y., 2005. Biosens. Bioelectron. 20 (7), 1407–1416.