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Table of contents

Page

Front cover	1
Standard Form (SF 298).....	2
Table of contents	3
Abstract	4
Introduction.....	4
Experimental Details.....	9
Protein and Antibody:	9
Materials	10
Experimental Procedures:	10
Characterization tools and methods.....	11
Results and Discussions.....	12
CNT based QCM sensor	16
Key research accomplishments.....	18
Reportable outcomes.....	18
Manuscripts, abstracts, presentations.....	18
Degrees obtained that are supported by this award	19
Conclusions.....	19
References.....	20
Collected Figure Captions.....	24
Appendices.....	40

Abstract

Acoustic wave sensors have been widely used for detection of various chemical and biological species in liquid media. An improved binding of Protein A and IgG molecules on QCM biosensors by modifying the gold surface of the quartz crystal with a 35nm polystyrene film followed by an acidic treatment was accomplished. The frequency shifts due to the binding of the Protein A and IgG were 220 Hz and 282 Hz respectively for direct binding onto the chip. There was an appreciable increase in the frequency shift when the polystyrene film was used as an interfacial layer. The shift with the polystyrene film for Protein A was 364 Hz (an increase of 65%) and for the IgG it was 391 Hz (an increase of 40%). Complementary Atomic Force Microscopy (AFM) studies were carried out to understand the parameters responsible for such improved biomolecular binding. AFM studies revealed a significant decrease in the RMS roughness of the substrate from 98.4 nm to 1.75 nm when coated with polystyrene resulting in higher antibody coverage on the surface of the sensor. This increased surface smoothness resulted in higher biomolecular coverage on the surface of the sensor causing higher frequency shifts.

The CNT novel nanostructure material was used as chemical interface to enhance the sensitivity of the acoustic wave sensors which resulted in five fold increase in the frequency shift. Immobilization of the fPSA at different concentrations on gold surface was achieved and corresponding sensor response were registered.

Introduction

According to the American Cancer Society Facts and Figures, there would be an estimated 230,110 new cases of prostate cancer in the year 2004 with around 29,900

deaths. Prostate cancer, therefore, is a considerable burden to not only those individuals that contract the disease but also to society as a whole. Being able to successfully treat prostate cancer would therefore have a significant impact on morbidity and mortality for a large group of individuals. Prostate specific antigen (PSA), a protein with a molecular weight of 33-34 KDa, has been found to be the most effective marker for diagnosis and detection of prostate cancer. Low concentrations of PSA would be detected using acoustic wave sensors because of their high sensitivity and ability to operate in a liquid environment.

Biosensors with rapid and highly sensitive detection capabilities of various biomolecules including PSA are of great demand in the field of life sciences. Existing immunoassay techniques such as enzyme linked immunoassay (ELISA), fluoroimmunoassay (FIA) and radioimmunoassay (RIA) require specific labels for detection purposes thus making them more complex and time consuming [1, 2, 3]. Further these techniques are cumbersome, laborious, expensive, and hazardous. Developing biosensors for detecting the concentration and activities of the various biological species therefore becomes very important. Recently acoustic wave sensors have been developed for the specific detection of various chemical and biological molecules in liquid media. The chemical interface selectively adsorbs materials in the solvent to the surface of the sensing area. Changes in the physical parameters of an acoustic device may perturb the mechanical properties of the wave and/or its associated electrical field. For biological sensors, binding of a substance onto the resonating membrane surface causes a decrease in the acoustic wave velocity, which is related to the resonant frequency of the device. The changes in the

acoustic and electromagnetic properties of the chip can be directly proportional to the changes in mass.

In this phase of the project in order to detect biomolecules, we have followed the following steps which are the achievements of the work to date:

- Preparation of protein chips for immobilization of various antibodies including Prostate Specific Antibodies.
- Studying the protein chips integrity in terms of uniformity and denaturation using the Atomic Force Microscopy technique.
- Exploration of various chemical interfaces like ultrathin polymer films and novel nanostructured materials to improve the sensitivity of gravimetric devices.
- Immobilization of Prostate Specific Antibodies on gold surfaces to construct the calibration curves for various concentrations of antibodies.

Since the required PSA detection limit is in the range of 4ng/ml to 20 ng/ml, we planned to develop a versatile technique(s) which can be employed for devices intended for our research. To this end we have chosen two methods for improvement of the sensitivity of the gravimetric devices. 1) Employing an ultrathin polymer film (35 nm thickness) and exploring the chemical modification required for biomolecule immobilization 2) utilizing carbon nanotubes as chemical interface by exploitation of hydrophobic interactions between the surface of CNT and biomolecules.

Quartz Crystal Microbalances (QCM) which is a Thickness Shear Mode (TSM) acoustic wave device was used to quantify and qualify the methodology described above. QCMs are based on these principles wherein a shift in the resonant frequency of the QCM can be attributed to the mass bound on the sensor membrane as per the Sauerbrey equation [4]

$$\Delta f = \frac{-2f_0^2 \Delta m}{A\sqrt{\rho_q \mu_q}} \quad (1)$$

where f_0 is the fundamental resonant frequency, A is the piezoelectrically active area defined by the two gold electrodes, ρ_q is the density of quartz (2.648 g cm^{-3}) and μ_q is the shear modulus ($2.947 \times 10^{11} \text{ dyn cm}^{-2}$). Equation (1) is based on an assumption that the mass has been rigidly attached to the crystal and has negligible thickness as compared to the crystal as a whole [4]. Under such conditions the thin film acts as an extension of the quartz thickness and experiences no shear force during the crystal oscillation [4].

Quartz Crystal Microbalances have been used extensively for protein sensing [5, 6] and gravimetric immunoassays [1, 2, 7, 8, 9]. The simple relationship between the change in frequency (Δf) and the change in mass (Δm) enables QCM to be widely used in sensing applications. Modification of QCM surface with various polymers like polyurethane [10, 11], polyethylene amine [12, 13, 14], and polystyrene [8, 15, 16] have been previously reported. Polystyrene (PS) is a very common polymer with a high processing ability due to its solubility in most of the organic solvents. The Young's modulus of PS is $\sim 3 \text{ GPa}$ as compared to $\sim 3 \text{ MPa}$ of polyurethane. Due to this high mechanical strength no significant viscoelastic coupling effects are observed between PS and the quartz crystal [17]. This particular property of PS makes it an ideal choice as an interfacial layer to improve the biomolecular binding. Previous reports on PS modified QCM surfaces involved

immobilization of biomolecules directly onto the polymer surface [8, 15, 16]. In this research work we have developed the silanization technique which improves the hydrophilicity of the PS surface by providing the terminal amine groups which in turn increase the number active sites of the surface for biomolecule immobilization.

Carbon nanotube is an interesting material at the intersection of bio- and nano-technologies. Although carbon nanotubes have many applications, one of the most important and promising applications which might find a significant place in the technologies related to these buckytubes is its biosensing ability [18, 19]. The unique electronic properties of CNT in conjunction with the specific recognition properties of the incubated biomolecules would make CNTs as ideal nanoscale biosensors. A careful observation reveals that carbon nanotubes have the large surface area with all the carbon atoms on the surface. Hence, by a careful alteration of the surface chemistry of the carbon nanotube, they can be exploited for biosensing applications [20] which is motivation behind using CNTs as a chemical interface on the mass sensitive devices. It has been recently demonstrated that individual semiconducting single wall carbon nanotubes can be used for the detection of glucose oxidase [21]. Controlled attachment of the glucose oxidase enzyme (GOx) to the SWNT sidewall was achieved through a linking molecule which resulted in a clear change of the conductance of the sensing device. We employed QCM to quantify the mass of the biomolecules bound on the surface of the nanotubes.

Historically antibodies have served as important diagnostic tools for the detection of diverse conditions and diseases including those that affect the thyroid, HIV, diabetes,

pregnancy, and several types of cancer. In clinical settings, antibodies have been used in several different assays including radioimmunoassays (RIAs), enzyme linked immunoassays (ELISAs) and Western blots. The utility of these assays relies on their ease of use, reproducibility, accuracy and relatively low cost. To expand the technology that is available for the use of antibody based assays we are developing an acoustic wave sensor for the detection of antibody-antigen complexes from complex mixtures. The advantages to this system will be that it is highly reproducible, inexpensive, relatively easy to use and highly portable and more sensitive than the assays in which antibodies are typically used. Although the structure of antibodies are obviously highly variable, they also contain regions of high similarity and the methods that we develop will rely on the interaction of the detection system with those regions of similarity for the detection of the antibody-antigen complexes. The technology that is created will therefore be readily transferable to the detection of other antibody-antigen complexes of clinical interest. Due to the prevalence and clinical relevance we chose to develop this technology for the detection of prostate specific antigen (PSA), a biomarker that has been used for the detection of prostate cancer in men. Increased concentration of this antigen has been correlated with the staging of prostate cancer and it has a long history of use in a clinical setting as a biomarker. We have immobilized various concentrations of Prostate Specific Antibodies on gold surfaces to construct the calibration curves of antibody.

Experimental Details

Protein and Antibody:

fPSA (BioDesign International Inc), Protein A (Sigma-Aldrich) and mouse monoclonal IgG antibody (BioDesign International Inc) were used throughout these studies. Protein

A, fPSA and monoclonal IgG were resuspended in phosphate buffered saline (PBS; Sigma-Aldrich) at desired concentrations and stored at -20°C in 50µl aliquots before use.

Materials

Polystyrene, 3-Aminopropyl triethoxysilane (3-APTES), glutaraldehyde, acetone, glycine and sodium chloride were purchased from Sigma-Aldrich Chemical Company. Polystyrene dissolved in chloroform (7% w/v) was used to coat the QCM chips. Solutions of 5% 3-APTES in acetone, 5% glutaraldehyde in milli-Q water, PBS buffer with pH 7.0 in milli-Q water were prepared. 0.1M Glycine solution in milli-Q water, 0.1M glycine-HCl buffer with pH 2.4 and 0.5M NaCl solution was prepared. CNTs were purchased from Carbolex Inc.

Experimental Procedures:

For promoting the immobilization of Protein A and to provide the necessary amine groups on the gold surface the protocol of Muramatsu et al. (1987) was followed [1]. To remove any organic contamination from the surface of the crystal and improve the hydrophilic nature of the chip, it was cleaned with Piranha solution (3 parts of H₂SO₄ in 1 part of 30 % H₂O₂). Enough Piranha solution was employed to cover the gold surface of the chip and allowed to incubate at room temperature for two minutes before rinsing with milli-Q water. This procedure was repeated twice. Subsequently the chip was blown dried in a stream of nitrogen gas. A 5% solution of 3-APTES in acetone was added to create a self-assembled monolayer (SAM). After 1 hour the sample was rinsed with milli-Q water after the APTES treatment to remove the physisorbed molecules. The chip was placed in

a 5% glutaraldehyde solution for 3 hours to allow for the cross linking between the chip and the Protein A. The crystal was then covered with 20 μ l solution of Protein A (0.5 mg/ml). After 1 hour the solution was removed and the crystal was subjected to several wash-dry cycles with milli-Q water until the QCM crystal reached its steady resonant frequency. The chip was then covered with 0.1 M glycine dissolved in PBS for 1 hour to block any sites not bound to Protein A on the glutaraldehyde modified chip. The chip was then rinsed with 0.1M glycine-HCl buffer (pH 2.4) to wash off any excess proteins or glycine before being thoroughly rinsed with milli-Q water. 20 μ l of the mouse monoclonal IgG solution was then incubated on the chip for 1 hour followed by rinsing with 0.5M NaCl to remove any non-specifically adsorbed antibody. For the experiments in which binding was measured with the polymer film, polystyrene was spin coated onto the chip at a speed of 1000 rpm and then treated with 50 % (v/v) HNO₃ in concentrated H₂SO₄ for 1 hour [22]. The substrate was then modified with 3-APTES followed by glutaraldehyde as described above.

Characterization tools and methods

A Quartz Crystal Microbalance (Stanford Research Systems QCM100) with 5MHz AT-cut quartz crystals (gold coated) was used to quantitatively study the ability to bind Protein A and mouse monoclonal IgG to the chip. The gold surface, which forms the active area for immobilization was 1.37cm² and the mass sensitivity of the crystal was 0.057 Hz/ng/cm². Frequency was monitored using a Stanford Research System Universal Time Interval Counter (Model No. SR620).

Qualitative studies were made using AFM (Themomicroscopes Inc.; Autoprobe CP Research machine) in non-contact mode. For AFM studies silicon substrates were used with the same modification techniques as those described above for the QCM chips. The AFM tips used for imaging were silicon with an approximate radius of curvature of 20nm. Biomolecular imaging was performed in non-contact mode. The AFM images were analyzed using image-processing software (IP 2.1) to calculate the RMS roughness value.

Results and Discussions

Protein A, which has a particularly high affinity for the F_c fragment of IgG, was immobilized first on the chips to prevent the random immobilization of the antibodies, maximizing the ability of the chip immobilized antibodies to bind to antigens [1, 2]. Figure 1 shows the QCM frequency response to Protein A immobilization without the polystyrene film. Point 1 refers to the point of addition of the Protein A containing solution to the chip. Point 2 indicates when the crystal was subjected to several wash-dry cycles and point 3 represents the frequency of crystal when Protein A was specifically bound on the surface. The frequency shift due to this direct binding was 220 Hz. From the Sauerbrey equation, this frequency shift corresponds to a 2.8 μg mass uptake.

To determine if antibodies could bind to the Protein A that had been immobilized, antibody containing solutions were incubated with the chips. In Figure 2 point 1 indicates the time at which the antibody containing solution was added to the crystal. The binding of the antibody to the immobilized Protein A caused a decrease in the resonant frequency

and stabilization occurred after 15 minutes. Point 2 represents the time when the crystal was rinsed with 0.5M NaCl to remove any non-specifically adsorbed IgG and point 3 corresponds to the final resonant frequency after the NaCl rinsing. The frequency shift for IgG immobilization was found to be 282 Hz which corresponds to a calculated mass change of 3.61 μ g.

To determine if covering the chips with a thin polymer film could also increase the efficiency of Protein A binding and hence improvement in antibody immobilization, we coated the surface of several chips with ultra thin film of polystyrene. However, polystyrene films are hydrophobic in nature causing the biomolecules to denature and hence lose their activity [23]. To avoid denaturation of the biomolecules, the polymer film functional groups such as amino, hydroxyl groups can be chemically added. This helps the biomolecules retain their activity as immobilization now takes place through the hydrophilic arms of the polymer film [24, 25]. To increase the hydrophilicity of the surface which would increase the ability to add the functional groups, the chips were subjected to an acidic treatment followed by aqueous silanization [22]. Figure 3 shows the schematic representation of the acidic treatment and the APTES modification of polystyrene. The acid treatment provides NO₂ groups and the APTES modification creates a polymer film with an amine group that can react with the glutaraldehyde used to covalently attach the biomolecules to the surface.

This improvement in the hydrophilicity was confirmed by monitoring the water distribution on polystyrene and APTES modified polystyrene surfaces. Figure 4.a and Figure 4.b show the water distribution images (recorded using a Digital Blue Computer

microscope) on polystyrene and modified polystyrene surfaces. As is evident from the images there is a decrease in the contact angle of about 34° for APTES modified polystyrene surfaces compared to the unmodified surface indicating improvement in hydrophilicity.

Figure 5 shows the AFM image of IgG immobilized on polystyrene coated surface. It can be seen that there is a uniform coverage of the antibody molecules of approximately 10 nm in size on the substrate. The AFM imaging performed two hours after the biomolecules immobilization revealed that the molecules still retain their characteristic "heart shape" proving that they still are not denatured.

The biomolecule immobilization on polystyrene coated surfaces was then quantitatively studied with QCM and compared to the immobilization performed without polystyrene film. Figure 6 shows the QCM response to Protein A immobilization. Point 1 indicates the time when Protein A was added and as can be seen the signal became stable only after 20 minutes. Point 2 represents when several wash-dry cycles were performed and point 3 is the time at which the frequency stabilized once all non-specifically adsorbed molecules were rinsed away. The registered frequency shift was 364 Hz which corresponds to a mass change of 4.66 μg . This represented a 65% increase when compared to the QCM chips that were not coated with the polymer film. Similar results were obtained for the binding of IgG. The QCM response (Figure 7) for IgG immobilization on the polystyrene surface showed a frequency shift of 391 Hz corresponding to a mass uptake of 5.01 μg .

This represented a 40% increase when compared to chips that had not been modified with polystyrene.

AFM images of the bare gold crystal and polystyrene coated crystal along with the height profile of the surface along a line are shown in Figure 8 and Figure 9 respectively. The AFM studies (as is evident from Figure 8) revealed that the gold coated quartz crystals had a RMS surface roughness of 98.4nm. An appreciable decrease in the surface roughness to 1.75nm was observed when the crystal was coated with an ultra thin layer of polystyrene. Gold and polystyrene are both hydrophobic in nature. APTES modification of the gold surface although improves the hydrophilicity of the surface, it doesn't result in much decrease in the roughness of the surface. On the other hand APTES modification of the polystyrene coated surface not only improves the hydrophilicity of the surface but there is a marked improvement in the surface roughness because of the polymer film. The improved biomolecular binding and hence the increased frequency shifts may be attributed to this improvement in the surface smoothness.

We speculate that with a gold surface roughness of 98.4nm, the orientation of the protein A molecules is not uniform and hence there are chances that the active sites on one protein molecule would sterically hinder the active sites on the other resulting in a non uniform binding of biomolecules and hence loss of active sites. On the other hand a polymer coated surface although decreases the available surface area, provides the biomolecules with a much more plane and uniform surface resulting in less steric hindrance. Hence more active sites for antibody immobilization are available resulting in improved binding and hence higher sensitivity.

CNT based QCM sensor

CNT experiments were performed using 5 MHz AT-cut Quartz crystals coated with carbon nanotubes casted on the gold surface of the chip. Then the chip was baked at 50°C for one hour. The presence of the CNT on the gold surface was confirmed by Raman spectroscopy. Figure 10 shows the Raman spectrum of CNT on the gold surface. The characteristic G-band and D-band peaks of the carbon nanotube can be observed. Figure 11 is an AFM micrograph of the CNT and protein molecules. From this Figure, it can be seen that protein molecules were bound on the sidewalls of the tube and bundles of CNT were decorated with Streptavidin molecules.

The quantitative study of mass uptake of CNT network due to biomolecules immobilization was performed using QCM. In this part, the chemical interface is the CNT matrix on the gold surface coated on the QCM crystal. Measurements of CNT coated QCM crystals were performed by covering the chips with PBS before addition of the protein solutions. Figure 12 depicts the QCM response using streptavidin. For a concentration of 1 μ M of streptavidin, a change of 120 Hz in resonant frequency was recorded. From the Sauerbrey equation the mass bound was calculated to be 1.538 μ g. when the concentration on the chip was increased to 2 μ M, the change in the frequency was found to be 26Hz. This corresponds to a mass uptake of 0.33 μ g. The lower frequency shift can be attributed to fewer active sites available for the protein molecules as described in the conductance based sensors.

Similar experiments were performed with IgG and the frequency change was 248Hz using a 2 μ M concentration. Figure 13 shows the QCM response for the IgG incubation.

When the crystal was resonating at its natural frequency, the carbon nanotube solution was introduced on to the chip. After the chloroform evaporated, the frequency stabilized and the CNT formed a uniform matrix on the surface. Then PBS was introduced on to the chip and due to change in the viscosity a frequency shift was recorded. After the frequency stabilized, finally the IgG antibody was introduced and the frequency change of 248 Hz was recorded for a concentration of 2 μ M. This frequency corresponds to mass of 3.17 μ g. The frequency shift was found to be approximately 50Hz for same concentration of IgG immobilized directly on the gold surface which suggests that there is a five fold increase in the number of biomolecules bound when CNT was used as chemical interface.

fPSA at different concentrations were incubated on the surface of QCM chips to construct the calibration curve of antibody. Figure 14 depicts the QCM measurement for antibody at a concentration of 80 μ g/ml. The frequency shift exhibited by the crystal at this concentration was around 90Hz. This corresponds to mass of 1.153 μ g. At a concentration of 120 μ g/ml a frequency shift of 120 Hz was registered (Figure 15). A frequency shift of 130 Hz was observed for antibody concentration of 160mg/ml. Mass of the antibody immobilized was found to be 1.66 μ g.

Key research accomplishments

Key research accomplishments of this work includes:

- 1) Ultrathin polystyrene polymer films can be used to enhance the sensitivity and these films can be chemically treated to provide necessary terminal groups to tether biomolecules to the surface.
- 2) A 40% to 60% improvement in sensor sensitivity was demonstrated with aqueous silanization was achieved.
- 3) Novel nanostructure materials like carbon nanotubes have been integrated with existing technologies to push the detection limit of acoustic wave based sensors.
- 4) CNT based interfacial layer showed five fold improvement of the sensor response.
- 5) fPSA antibody at different concentrations was immobilized on the gold surface.

Reportable outcomes

Manuscripts, abstracts, presentations

1. M.Z. Atashbar, B. Bejcek, A. Vijh, and S. Singamaneni "QCM Biosensor with ultra thin polymer film", Sensor and Actuators B (in press, available online).
2. M.Z. Atashbar, B. Bejcek, and S. Singamaneni "SWNT Network for biomolecule detection", MRS Fall Meeting, November 2004, Boston (in press).
3. M.Z. Atashbar, B. Bejcek, S. Singamaneni "Carbon nanotube based biosensors", IEEE Sensor conference, Vienna, Austria, Oct. 24th-27th, 2004 (in press).
4. M.Z. Atashbar, B. Bejcek, A. Vijh, S. Singamaneni "Sensitivity enhancement of QCM biosenor with polymer treatment", 2004 IEEE International Ultrasonics, Ferroelectrics, and Frequency Control, 23-27th August 2004 pp. 329-332, Monntreal, Canada (2004).

5. M.Z. Atashbar, B. Bejcek, A. Vijh, and S. Singamaneni "Piezoelectric biosensor for prostate cancer detection", 4th Annual Symposium of Michigan Prostate Research Colloquium, Van Andel Research Institute, Grand Rapids, MI, May 1, (2004).

Degrees obtained that are supported by this award

1. Mr. Srikant Signamanieni, MSc. in Electrical Engineering, December 2004
2. Mr. Aditya Vijh, MSc. in Electrical Engineering, December 2004

Conclusions

Owing to the increasing demands in consumer, medical and military field's development of the highly sensitive biosensors has gained utmost importance. It was demonstrated that the treatment of the QCM quartz chips with polystyrene exhibited a significant decrease in the RMS roughness of the gold crystal, resulting in a surface that was more uniform. The disadvantage of this technique, however, is that the hydrophobicity of the surface remains high. To counteract this undesirable trait, chips were treated by a simple aqueous silanization technique, which resulted in a significant improvement in the hydrophilicity of the surface. Chips that had an improved hydrophilicity were successfully utilized for biomolecular immobilization. Protein A resulted in a shift of 364 Hz on the polystyrene modified surface as opposed to the 220 Hz shift of frequency for direct immobilization. Subsequent IgG immobilizations also showed similar trend with the frequency shifts being 391Hz and 282 Hz for immobilizations with and without the polymer film respectively. AFM imaging revealed uniform biomolecule coverage on

polymer coated surfaces. This improved binding of biomolecules when coated with polystyrene is hypothesized to be due to the marked improvement in the roughness of the surface over which the biomolecules were immobilized.

The biomolecules were adsorbed on the sidewalls of carbon nanotube. The mass of the biomolecule bound was quantitatively analyzed using QCM and CNT coated crystals exhibited a five fold increase in biomolecule adsorption. In summary one can conclude that carbon nanotube can find significant applications in the new generation of biosensors. Functionalization of carbon nanotube might significantly improve the chemical compatibility and selectivity of the sensors. Immobilization of fPSA on gold surfaces was accomplished and the methodologies derived for sensitivity enhancement will be utilized to target 4 ng/ml detection of fPSA using acoustic wave devices such as FPW and SH-SAW sensors.

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Collected Figure Captions

Figure 1. QCM frequency response for Protein A immobilization without polystyrene.

Figure 2. QCM frequency response for IgG immobilization without polystyrene.

Figure 3. Polystyrene film when treated with acid and 3-APTES; (a) Polystyrene film (b) Formation of the NO₂ groups by acidic treatment (c) Formation of the amine groups by silanization.

Figure 4. Image of water distribution on polystyrene (a) and APTES modified polystyrene (b) surfaces.

Figure 5. AFM image of IgG immobilized on polystyrene coated surface (b) height profile of the surface along a line.

Figure 6. QCM frequency response for Protein A immobilization on polystyrene

Figure 7. QCM frequency response for IgG immobilization on polystyrene.

Figure 8. (a) AFM image of the gold surface and the (b) height profile of the surface along a line.

Figure 9. (a) AFM image of polystyrene coated gold surface and (b) the height profile of the polystyrene surface along a line.

Figure 10. Raman spectrum of gold surface of QCM casted with SWNT.

Figure 11. Streptavidin molecules decorating the SWNT side walls.

Figure 12. QCM response for various concentrations streptavidin.

Figure 13. QCM response for various concentrations of IgG.

Figure 14. Frequency shift for fPSA antibody (80µg/ml) immobilization.

Figure 15. Frequency shift for fPSA antibody (120µg/ml) immobilization.

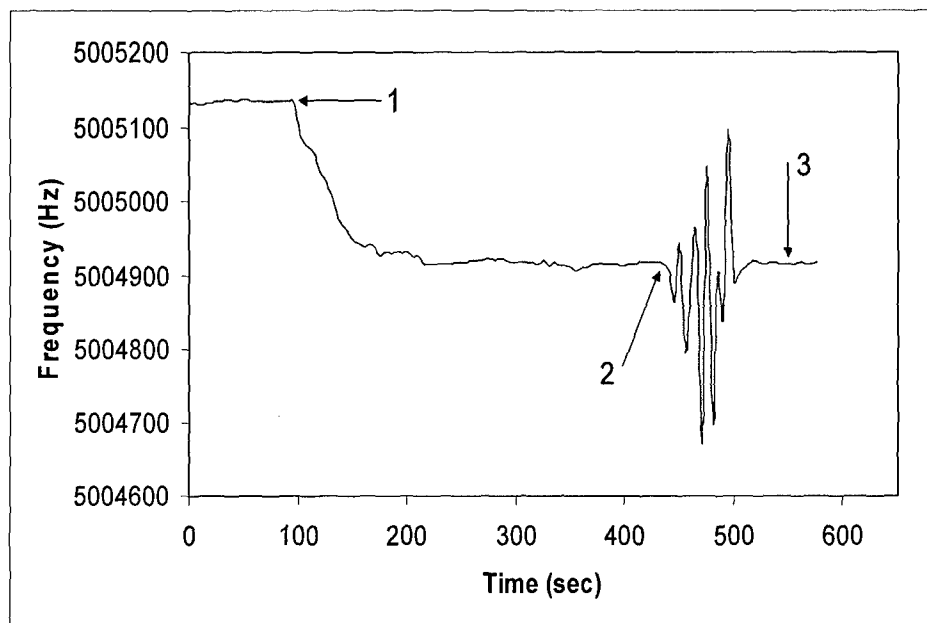


Figure 1. QCM frequency response for Protein A immobilization without polystyrene.

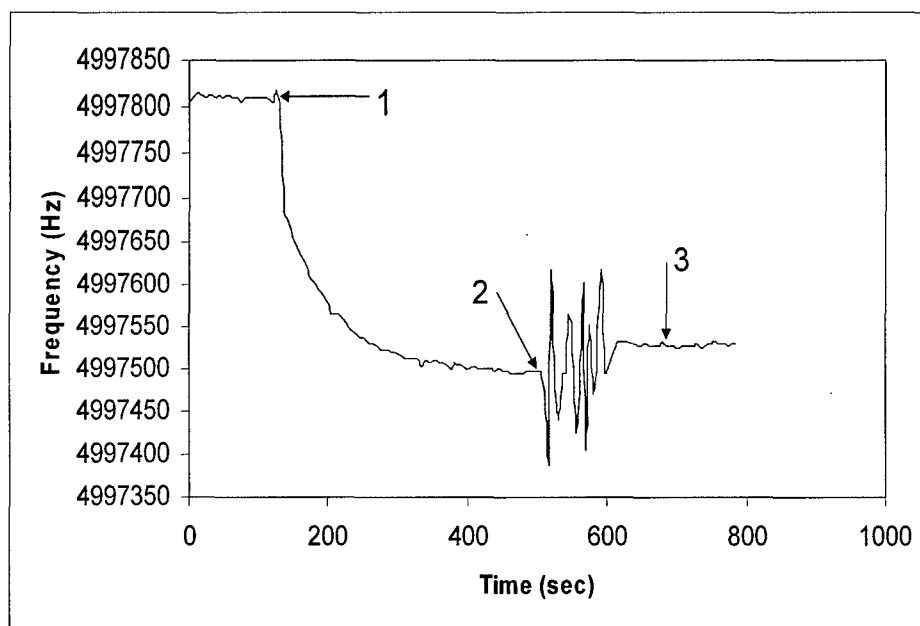


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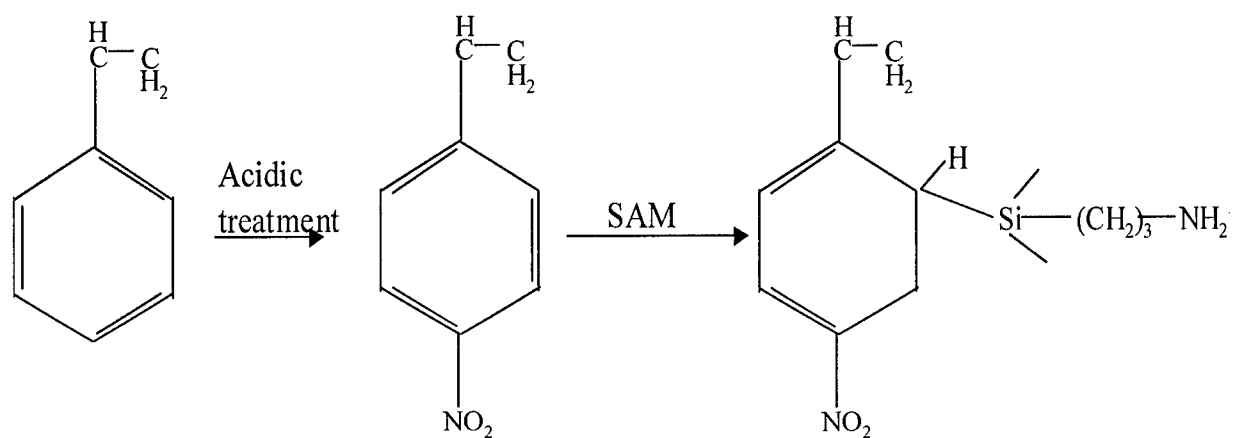
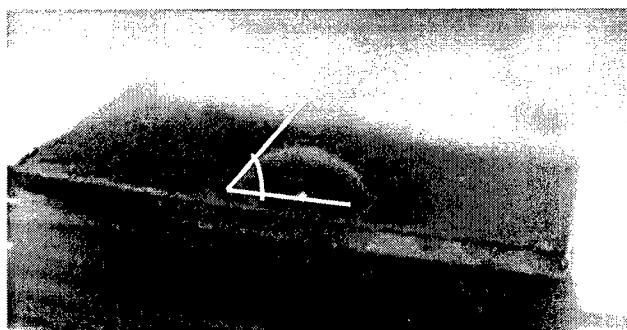


Figure 3. Polystyrene film when treated with acid and 3-APTES; (a) Polystyrene film (b) formation of the NO_2 groups by acidic treatment (c) formation of the amine groups by silanization.

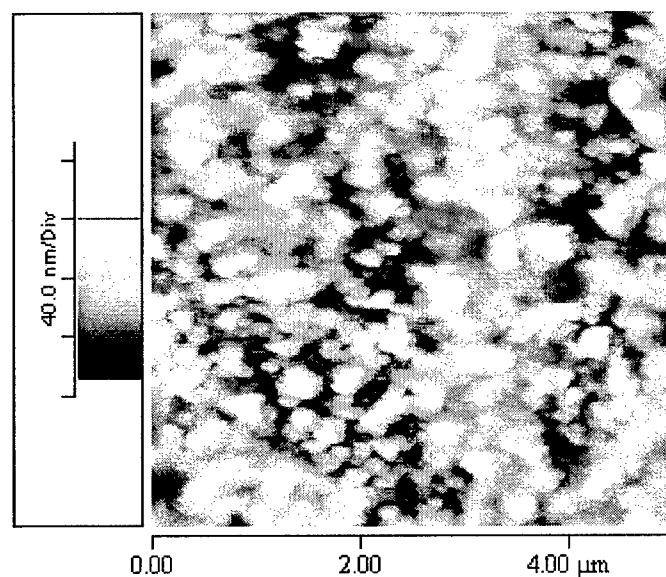


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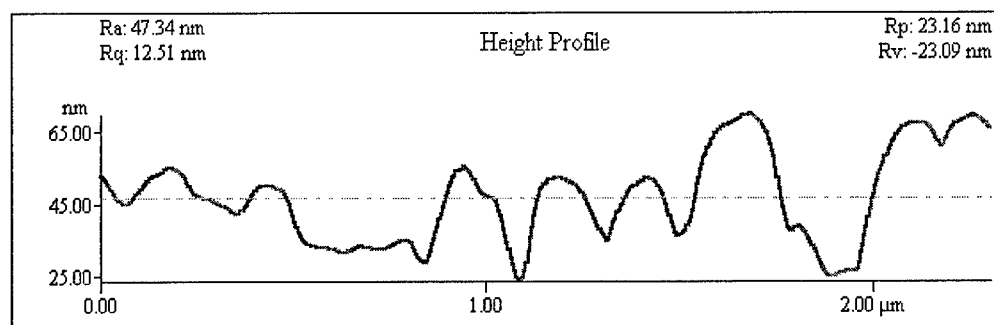


(b)

Figure 4. Image of water distribution on polystyrene (a) and APTES modified polystyrene (b) surfaces.



(a)



(b)

Figure 5. AFM image of IgG immobilized on polystyrene coated surface (b) height profile of the surface along a line.

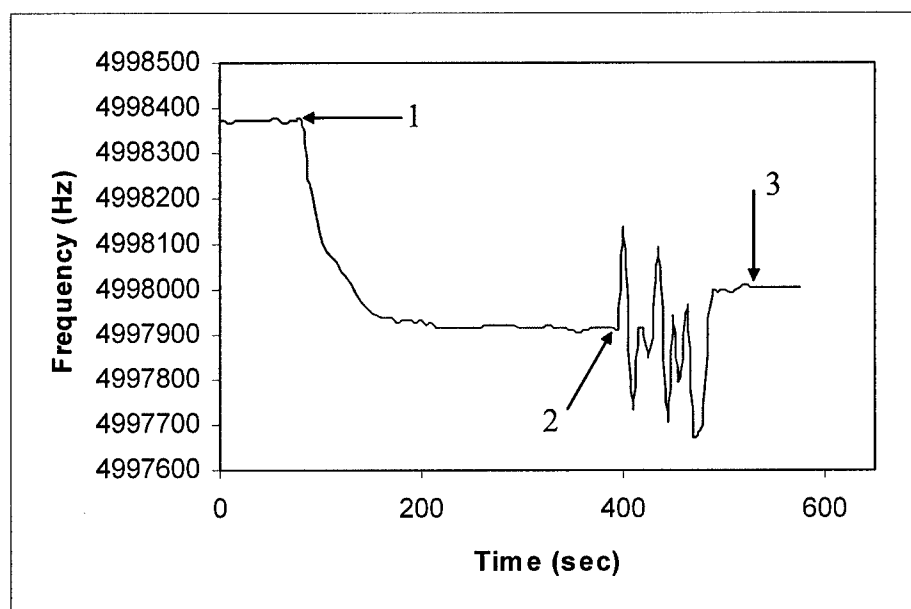


Figure 6. QCM frequency response for Protein A immobilization on polystyrene.

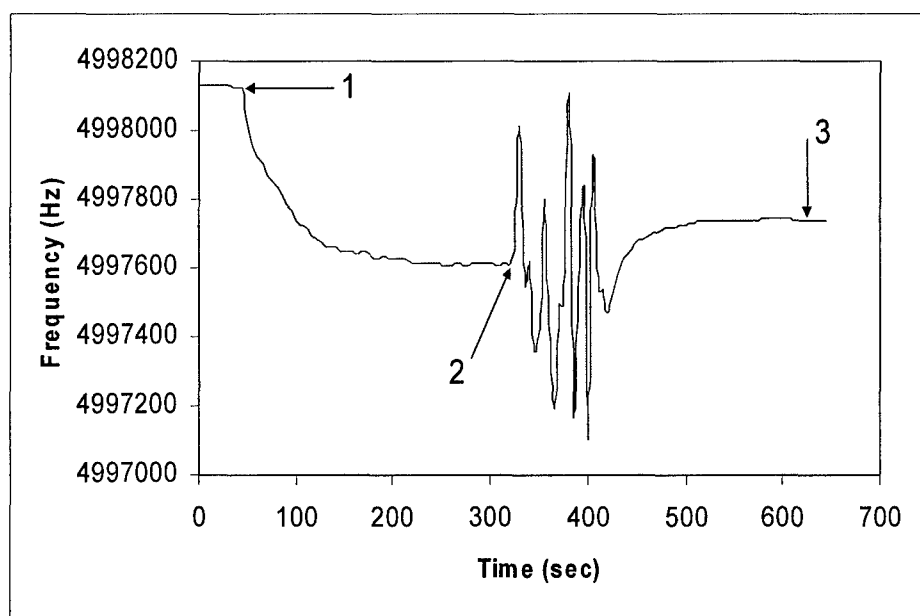
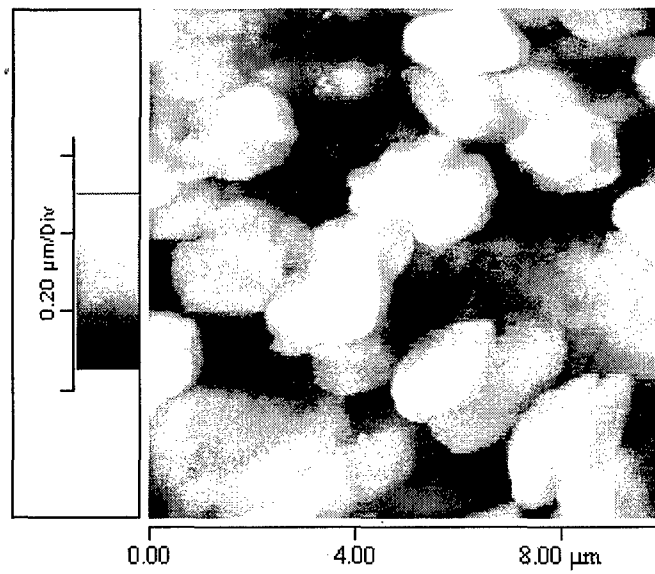
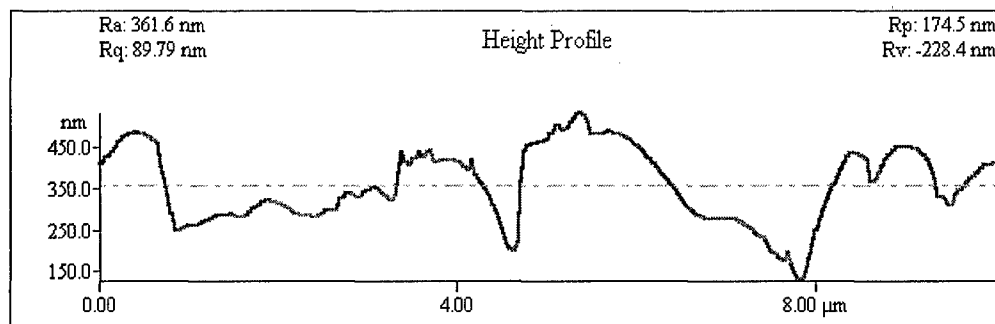


Figure 7. QCM frequency response for IgG immobilization on polystyrene.

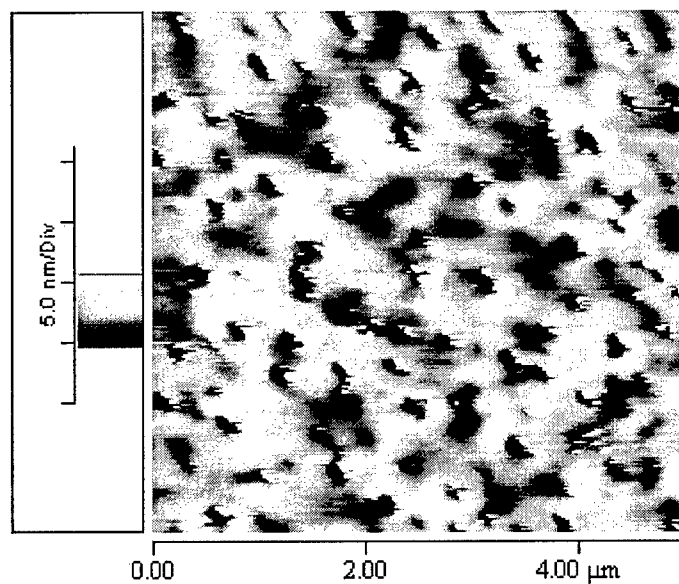


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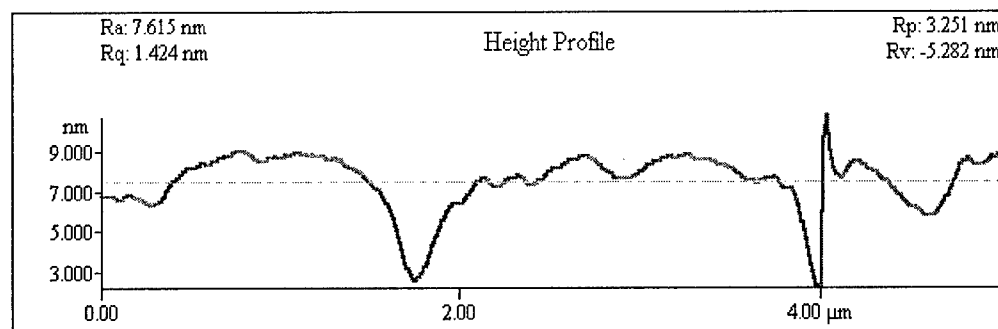


(b)

Figure 8: (a) AFM image of the gold surface of the QCM chip (b) height profile of the surface along a line.



(a)



(b)

Figure 9: AFM image of polystyrene coated gold surface and (b) the height profile of the polystyrene surface along a line.

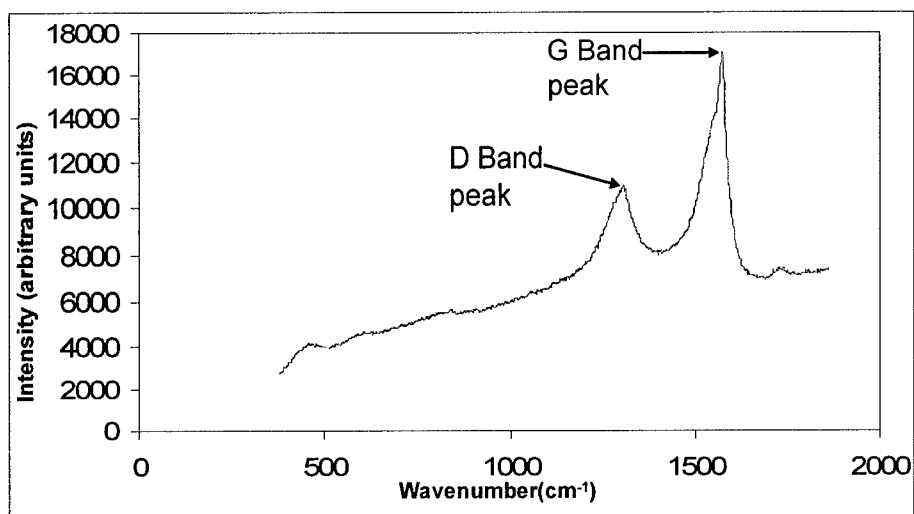


Figure 10. Raman spectrum of gold surface of QCM casted with SWNT.



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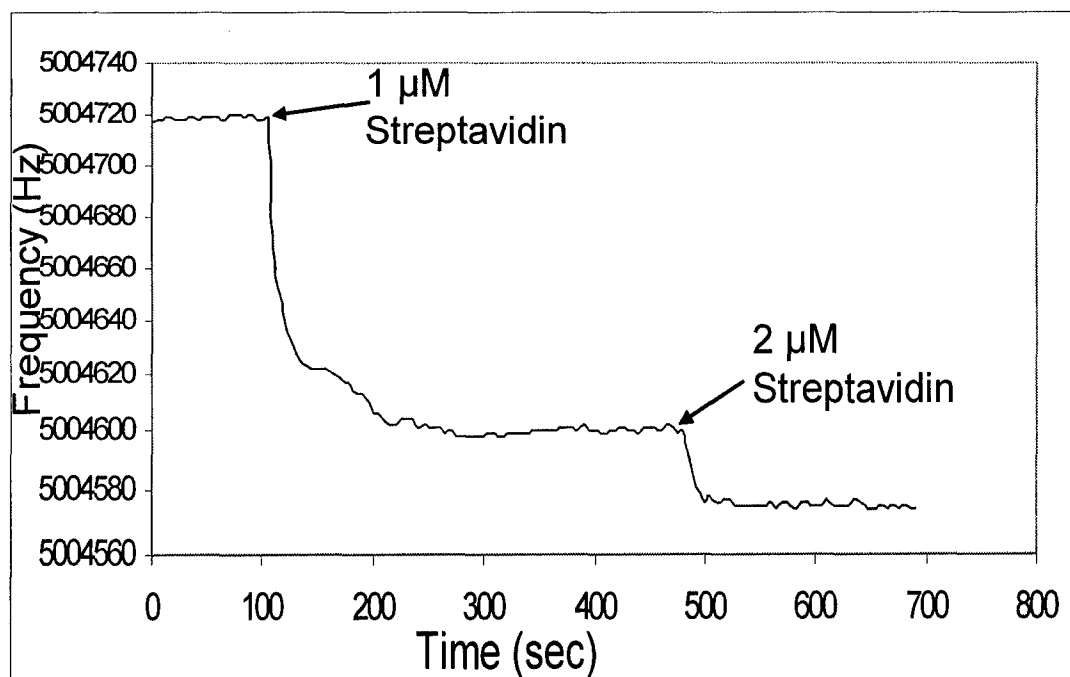


Figure 12. QCM response for various concentrations streptavidin.

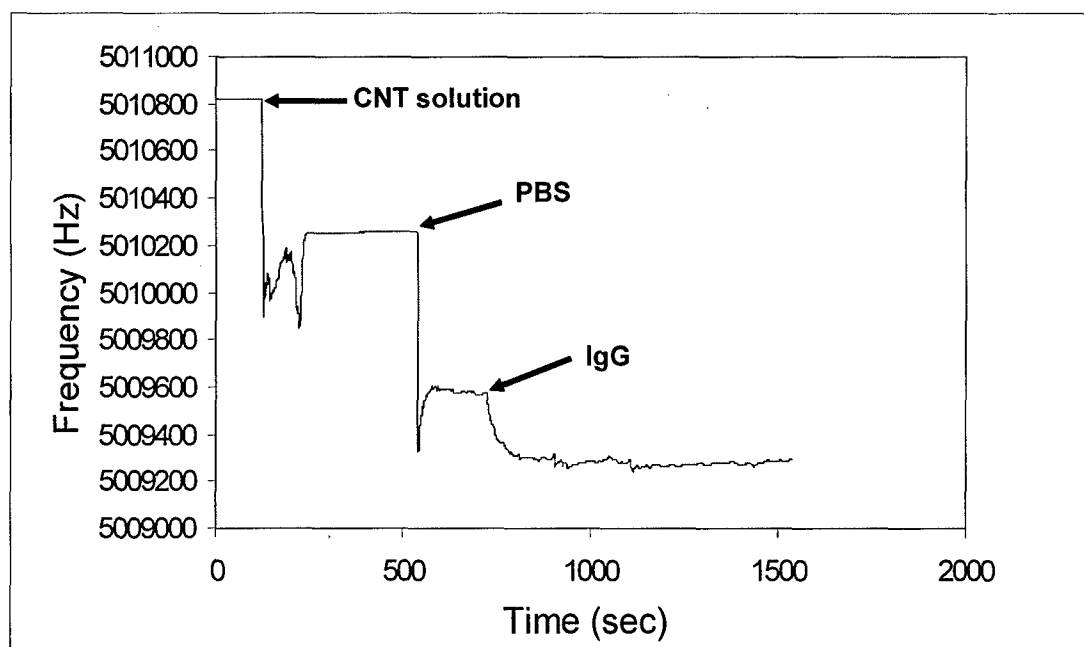


Figure 13. QCM response for various concentrations of IgG.

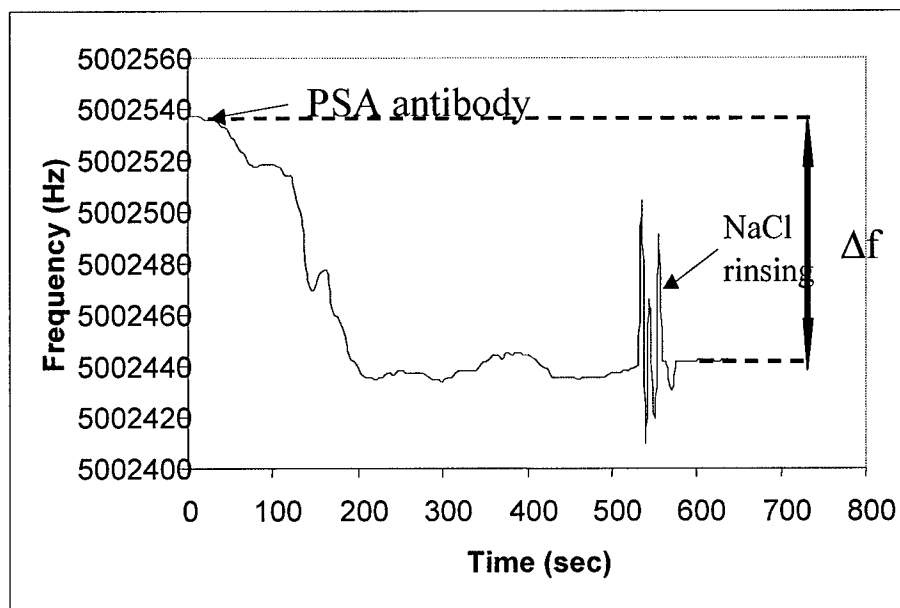


Figure 14. Frequency shift for fPSA antibody (80 μ g/ml) immobilization.

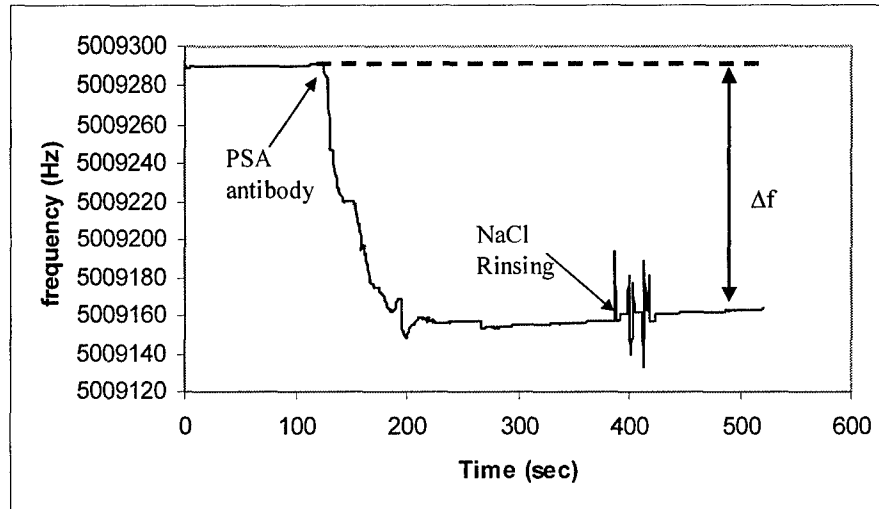


Figure 15. Frequency shift for fPSA antibody (120 μ g/ml) immobilization.

Appendices

- 1) M.Z. Atashbar, B. Bejcek, A. Vijh, and S. Singamaneni "QCM Biosensor with ultra thin polymer film", Sensor and Actuators B (in press, available online).
- 2) M.Z. Atashbar, B. Bejcek, and S. Singamaneni "SWNT Network for biomolecule detection", MRS Fall Meeting, November 2004, Boston (in press).
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QCM biosensor with ultra thin polymer film

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Abstract

Acoustic wave sensors have been widely used for detection of various chemical and biological species in liquid media. We report an improved binding of Protein A and IgG molecules on QCM biosensors by modifying the gold surface of the quartz crystal with a 35 nm polystyrene film followed by an acidic treatment. The frequency shifts due to the binding of the Protein A and IgG were 220 and 282 Hz, respectively for direct binding onto the chip. There was an appreciable increase in the frequency shift when the polystyrene film was used as an interfacial layer. The shift with the polystyrene film for Protein A was 364 Hz (an increase of 65%) and for the IgG it was 391 Hz (an increase of 40%). Complementary Atomic Force Microscopy (AFM) studies were carried out to understand the parameters responsible for such improved biomolecular binding. AFM studies revealed a significant decrease in the RMS roughness of the substrate from 98.4 to 1.75 nm when coated with polystyrene resulting in higher antibody coverage on the surface of the sensor. This increased surface smoothness resulted in higher biomolecular coverage on the surface of the sensor causing higher frequency shifts.

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Keywords: Acoustic wave sensors; Quartz crystal microbalance; Ultra thin polystyrene film; Atomic force microscope

1. Introduction

Biosensors with rapid and highly sensitive detection capabilities of various biomolecules are of great demand in the field of life sciences. Existing immunoassay techniques such as enzyme linked immunoassay (ELISA), fluoroimmunoassay (FIA) and radioimmunoassay (RIA) are cumbersome, laborious, expensive, and hazardous and require specific labels for detection purposes thus making them more complex and time consuming [1–3]. Developing biosensors for detecting the concentration and activities of the various biological species therefore becomes very important. Recently acoustic wave sensors have been developed for the specific detection of various chemical and biological molecules in liquid media. The chemical interface selectively adsorbs materials in

the solvent to the surface of the sensing area. Changes in the physical parameters of an acoustic device may perturb the mechanical properties of the wave and/or its associated electrical field. For biological sensors, binding of a substance onto the resonating membrane surface causes a decrease in the acoustic wave velocity, which is related to the resonant frequency of the device. The changes in the acoustic and electromagnetic properties of the chip can be directly proportional to the changes in mass.

Quartz Crystal Microbalances (QCMs) are based on these principles wherein a shift in the resonant frequency of the QCM can be attributed to the mass bound on the sensor membrane as per the Sauerbrey equation [4]

$$\Delta f = \frac{-2f_0^2 \Delta m}{A \sqrt{\rho_q \mu_q}} \quad (1)$$

where f_0 is the fundamental resonant frequency, A the piezoelectrically active area defined by the two gold electrodes, ρ_q the density of quartz (2.648 g cm^{-3}) and μ_q the shear modu-

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lus ($2.947 \times 10^{11} \text{ dyn cm}^{-2}$). Eq. (1) is based on an assumption that the mass has been rigidly attached to the crystal and has negligible thickness as compared to the crystal as a whole [4]. Under such conditions the thin film acts as an extension of the quartz thickness and experiences no shear force during the crystal oscillation [4].

Quartz Crystal Microbalances have been used extensively for protein sensing [5,6] and gravimetric immunoassays [1,2,7–9]. The simple relationship between the change in frequency (Δf) and the change in mass (Δm) enables QCM to be widely used in sensing applications. Modification of QCM surface with various polymers like polyurethane [10,11], polyethylene amine [12–14], and polystyrene [8,15,16] have been previously reported. Polystyrene (PS) is a very common polymer with a high processing ability due to its solubility in most of the organic solvents. The Young's modulus of PS is $\sim 3 \text{ GPa}$ as compared to $\sim 3 \text{ MPa}$ of polyurethane. Due to this high mechanical strength no significant viscoelastic coupling effects are observed between PS and the quartz crystal [17]. This particular property of PS makes it an ideal choice as an interfacial layer to improve the biomolecular binding. Previous reports on PS modified QCM surfaces involved immobilization of biomolecules directly onto the polymer surface [8,15,16]. In this work we report a silanization technique which improves the hydrophilicity of the PS surface by providing the terminal amine groups.

2. Experimental details

2.1. Protein and antibody

Protein A (Sigma–Aldrich) and mouse monoclonal IgG antibody (BioDesign International Inc.) were used throughout these studies. Protein A was resuspended in phosphate buffered saline (PBS; Sigma–Aldrich) at a concentration of $500 \mu\text{g/ml}$ and the mouse monoclonal IgG was resuspended in PBS at a concentration of $160 \mu\text{g/ml}$ and stored at -20°C in $50 \mu\text{l}$ aliquots before use.

2.2. Materials

Polystyrene, 3-aminopropyl triethoxysilane (3-APTES), glutaraldehyde, acetone, glycine and sodium chloride were purchased from Sigma–Aldrich Chemical Company. Polystyrene dissolved in chloroform (7%, w/v) was used to coat the QCM chips. Solutions of 5% 3-APTES in acetone, 5% glutaraldehyde in milli-Q water, PBS buffer with pH 7.0 in milli-Q water were prepared. 0.1 M Glycine solution in milli-Q water, 0.1 M glycine–HCl buffer with pH 2.4 and 0.5 M NaCl solution was prepared.

2.3. Experimental procedures

For promoting the immobilization of Protein A and to provide the necessary amine groups on the gold surface

the protocol of Muramatsu et al. was followed [1]. To remove any organic contamination from the surface of the crystal and improve the hydrophilic nature of the chip, it was cleaned with Piranha solution (three parts of H_2SO_4 in one part of 30% H_2O_2). Enough Piranha solution was employed to cover the gold surface of the chip and allowed to incubate at room temperature for two minutes before rinsing with milli-Q water. This procedure was repeated twice. Subsequently the chip was blown dried in a stream of nitrogen gas. A 5% solution of 3-APTES in acetone was added to create a self-assembled monolayer (SAM). After 1 h the sample was rinsed with milli-Q water after the APTES treatment to remove the physisorbed molecules. The chip was placed in a 5% glutaraldehyde solution for 3 h to allow for the cross linking between the chip and the Protein A. The crystal was then covered with $20 \mu\text{l}$ solution of Protein A (0.5 mg/ml). After 1 h the solution was removed and the crystal was subjected to several wash–dry cycles with milli-Q water until the QCM crystal reached its steady resonant frequency. The chip was then covered with 0.1 M glycine dissolved in PBS for 1 h to block any sites not bound to Protein A on the glutaraldehyde modified chip. The chip was then rinsed with 0.1 M glycine–HCl buffer (pH 2.4) to wash off any excess proteins or glycine before being thoroughly rinsed with milli-Q water. $20 \mu\text{l}$ of the mouse monoclonal IgG solution was then incubated on the chip for 1 h followed by rinsing with 0.5 M NaCl to remove any non-specifically adsorbed antibody. For the experiments in which binding was measured with the polymer film, polystyrene was spin coated onto the chip at a speed of 1000 rpm and then treated with 50% (v/v) HNO_3 in concentrated H_2SO_4 for 1 h [18]. The substrate was then modified with 3-APTES followed by glutaraldehyde as described above.

2.4. Characterization tools and methods

A Quartz Crystal Microbalance (Stanford Research Systems QCM100) with 5 MHz AT-cut quartz crystals (gold coated) was used to quantitatively study the ability to bind Protein A and mouse monoclonal IgG to the chip. The gold surface, which forms the active area for immobilization was 1.37 cm^2 and the mass sensitivity of the crystal was 0.057 Hz/ng/cm^2 . Frequency was monitored using a Stanford Research System Universal Time Interval Counter (Model No. SR620).

Qualitative studies were made using AFM (Themomicroscopes Inc.; Autoprobe CP Research machine) in non-contact mode. For AFM studies silicon substrates were used with the same modification techniques as those described above for the QCM chips. The AFM tips used for imaging were silicon with an approximate radius of curvature of 20 nm. Biomolecular imaging was performed in non-contact mode. The AFM images were analyzed using image-processing software (IP 2.1) to calculate the RMS roughness value.

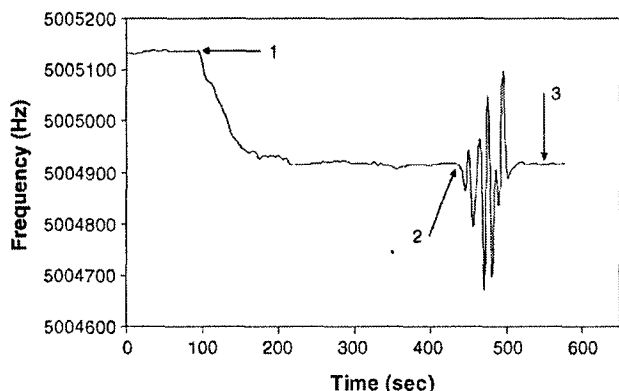


Fig. 1. QCM frequency response for Protein A immobilization without polystyrene.

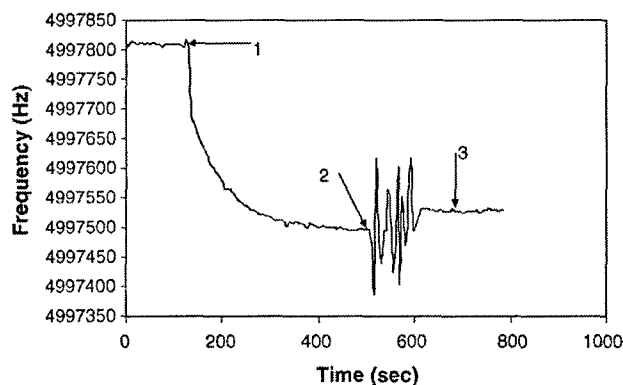


Fig. 2. QCM frequency response for IgG immobilization without polystyrene.

3. Results and discussions

Protein A, which has a particularly high affinity for the F_c fragment of IgG, was immobilized first on the chips to prevent the random immobilization of the antibodies, maximizing the ability of the chip immobilized antibodies to bind to antigens [1,2]. Fig. 1 shows the QCM frequency response to Protein A immobilization without the polystyrene film. Point 1 refers to the point of addition of the Protein A containing solution to the chip. Point 2 indicates when the crystal was subjected to several wash–dry cycles and point 3 represents the frequency of crystal when Protein A was specifically bound on the surface. The frequency shift due to this direct binding was 220 Hz. From the Sauerbrey equation, this frequency shift corresponds to a 2.8 μg mass uptake.

To determine if antibodies could bind to the Protein A that had been immobilized, antibody-containing solutions were incubated with the chips. In Fig. 2 point 1 indicates the time at which the antibody-containing solution was added to the crystal. The binding of the antibody to the immobilized Protein A caused a decrease in the resonant frequency and stabilization occurred after 15 min. Point 2 represents the time when the crystal was rinsed with 0.5 M NaCl to remove any non-specifically adsorbed IgG and point 3 corresponds to the final resonant frequency after the NaCl rinsing. The fre-

quency shift for IgG immobilization was found to be 282 Hz, which corresponds to a calculated mass change of 3.61 μg .

To determine if covering the chips with a thin polymer film could also increase the efficiency of Protein A binding and hence improvement in antibody immobilization, we coated the surface of several chips with ultra thin film of polystyrene. However, polystyrene films are hydrophobic in nature causing the biomolecules to denature and hence loose their activity [19]. To avoid denaturation of the biomolecules, the polymer film functional groups such as amino, hydroxyl groups can be chemically added. This helps the biomolecules retain their activity as immobilization now takes place through the hydrophilic arms of the polymer film [20,21]. To increase the hydrophilicity of the surface, which would increase the ability to add the functional groups, the chips were subjected to an acidic treatment followed by aqueous silanization [18]. Fig. 3 shows the schematic representation of the acidic treatment and the APTES modification of polystyrene. The acid treatment provides NO_2 groups and the APTES modification creates a polymer film with an amine group that can react with the glutaraldehyde used to covalently attach the biomolecules to the surface.

This improvement in the hydrophilicity was confirmed by monitoring the water distribution on polystyrene and APTES modified polystyrene surfaces. Fig. 4a and b shows the water

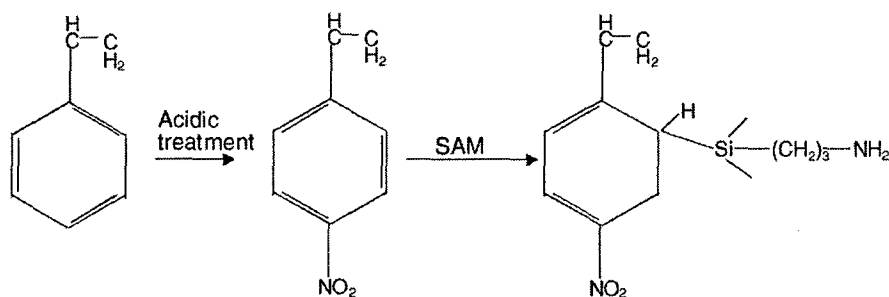


Fig. 3. Polystyrene film when treated with acid and 3-APTES: (a) polystyrene film; (b) formation of the NO_2 groups by acidic treatment; (c) formation of the amine groups by silanization.

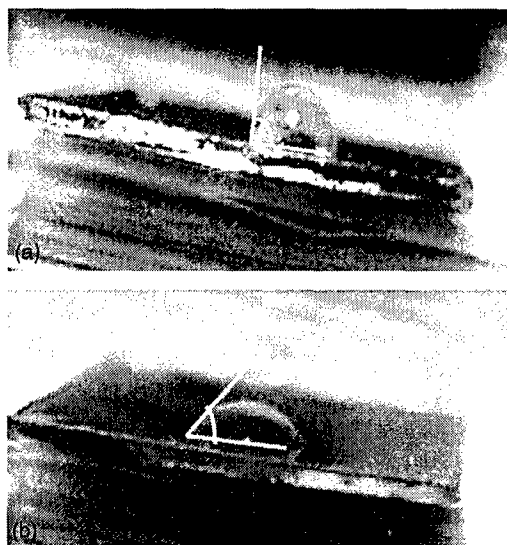


Fig. 4. Image of water distribution on polystyrene (a) and APTES modified polystyrene (b) surfaces.

distribution images (recorded using a Digital Blue Computer microscope) on polystyrene and modified polystyrene surfaces. As is evident from the images there is a decrease in the contact angle of about 34° for APTES modified polystyrene surfaces compared to the unmodified surface indicating improvement in hydrophilicity.

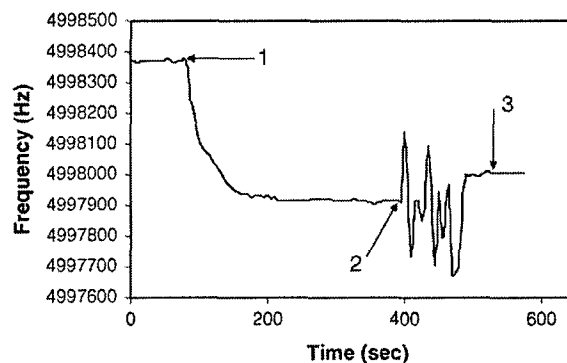


Fig. 6. QCM frequency response for Protein A immobilization on polystyrene.

Fig. 5 shows the AFM image of IgG immobilized on polystyrene coated surface. It can be seen that there is a uniform coverage of the antibody molecules of approximately 10 nm in size on the substrate. The AFM imaging performed 2 h after the biomolecules immobilization revealed that the molecules still retain their characteristic “heart shape” proving that they still are not denatured.

The biomolecule immobilization on polystyrene coated surfaces was then quantitatively studied with QCM and compared to the immobilization performed without polystyrene. Fig. 6 shows the QCM response to Protein A immobilization. Point 1 indicates the time when Protein A was added and as

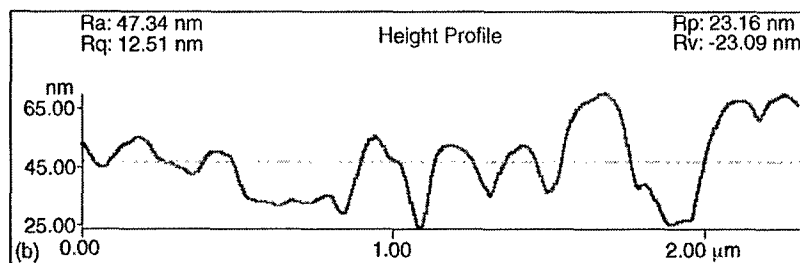
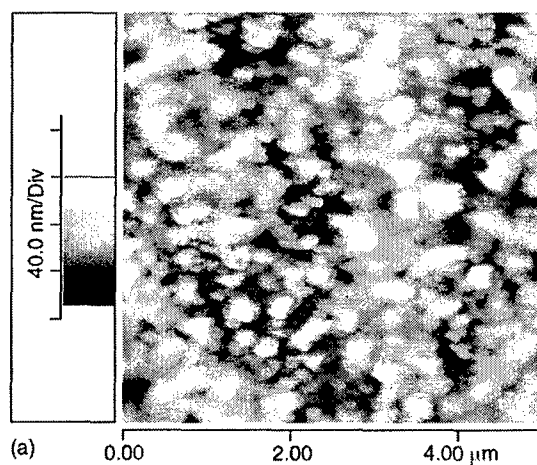


Fig. 5. AFM image of IgG immobilized on polystyrene coated surface and (b) the height profile of the surface along a line.

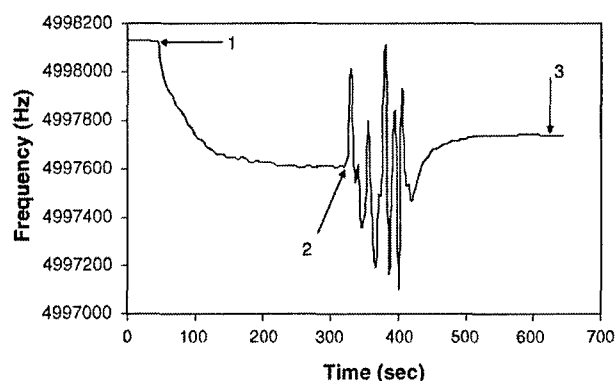


Fig. 7. QCM frequency response for IgG immobilization on polystyrene.

can be seen the signal became stable only after 20 min. Point 2 represents when several wash–dry cycles were performed and point 3 is the time at which the frequency stabilized once all non-specifically adsorbed molecules were rinsed away. The registered frequency shift was 364 Hz, which corresponds to a mass change of 4.66 μg . This represented a 65% increase when compared to the QCM chips that were not coated with the polymer film. Similar results were obtained for the binding of IgG. The QCM response (Fig. 7) for IgG immobilization on the polystyrene surface showed a frequency shift of 391 Hz corresponding to a mass uptake of 5.01 μg . This represented a 40% increase when compared to chips that had not been modified with polystyrene.

AFM images of the bare gold crystal and polystyrene coated crystal along with the height profile of the surface along a line are shown in Figs. 8 and 9, respectively. The AFM studies (as is evident from Fig. 8) revealed that the gold coated quartz crystals had a RMS surface roughness of 98.4 nm. An appreciable decrease in the surface roughness to 1.75 nm was observed when the crystal was coated with an ultra thin layer of polystyrene. Gold and polystyrene are both hydrophobic in nature. APTES modification of the gold surface although improves the hydrophilicity of the surface, it doesn't result in much decrease in the roughness of the surface. On the other hand APTES modification of the polystyrene coated surface not only improves the hydrophilicity of the surface but there is a marked improvement in the surface roughness because of the polymer film. The improved biomolecular binding and hence the increased frequency shifts may be attributed to this improvement in the surface smoothness.

We speculate that with a gold surface roughness of 98.4 nm, the orientation of the protein A molecules is not uniform and hence there are chances that the active sites on one protein molecule would sterically hinder the active sites on the other resulting in a non uniform binding of biomolecules and hence loss of active sites. On the other hand a polymer coated surface although decreases the available surface area, provides the biomolecules with a much more plane and uniform surface resulting in less steric hindrance. Hence more active sites for antibody immobilization

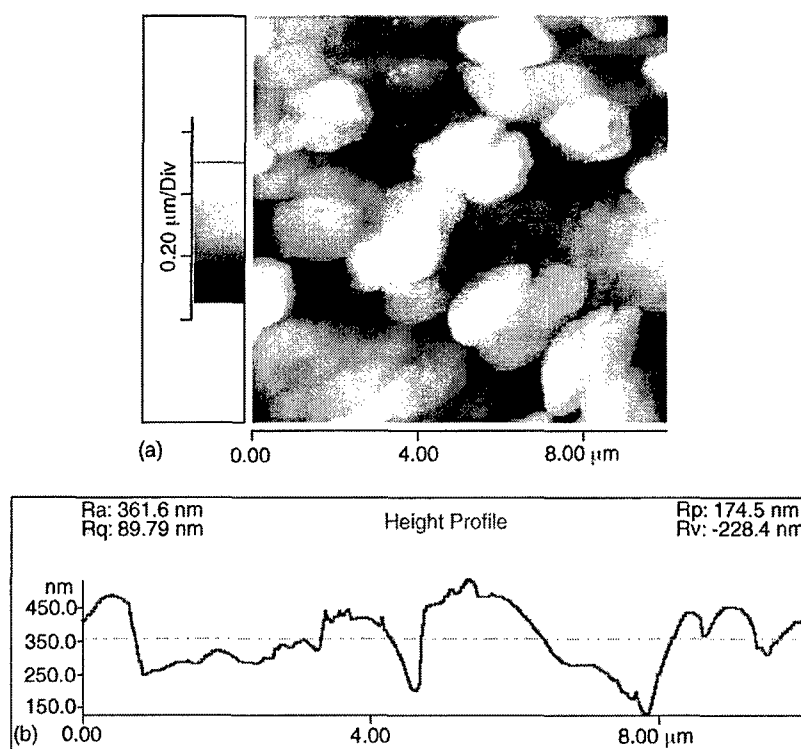


Fig. 8. (a) AFM image of the gold surface and (b) the height profile of the surface along a line.

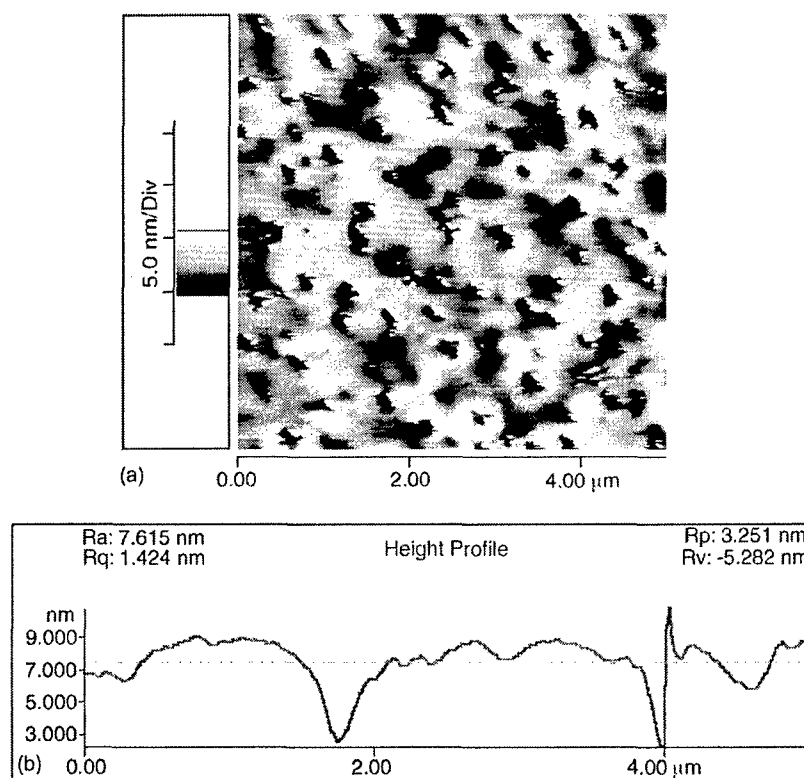


Fig. 9. (a) AFM image of polystyrene coated gold surface and (b) the height profile of the polystyrene surface along a line.

are available resulting in improved binding and hence higher sensitivity.

4. Conclusions

Owing to the increasing demands in consumer, medical and military field's development of the highly sensitive biosensors has gained utmost importance. It was demonstrated that the treatment of the QCM quartz chips with polystyrene exhibited a significant decrease in the RMS roughness of the gold crystal, resulting in a surface that was more uniform. The disadvantage of this technique, however, is that the hydrophobicity of the surface remains high. To counteract this undesirable trait, chips were treated by a simple aqueous silanization technique, which resulted in a significant improvement in the hydrophilicity of the surface. Chips that had an improved hydrophilicity were successfully utilized for biomolecular immobilization. Protein A resulted in a shift of 364 Hz on the polystyrene modified surface as opposed to the 220 Hz shift of frequency for direct immobilization. Subsequent IgG immobilizations also showed similar trend with the frequency shifts being 391 and 282 Hz for immobilizations with and without the polymer film, respectively. AFM imaging revealed uniform biomolecule coverage on polymer coated surfaces. This improved binding of biomolecules when coated with polystyrene is hypothesized to be due to the marked improvement in the

roughness of the surface over which the biomolecules were immobilized.

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SWNT NETWORK for BIOMOLECULE DETECTION

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ABSTRACT

In this paper we describe a single wall carbon nanotube (SWNT) based biological sensor for the detection of biomolecules using streptavidin and IgG. Two types of sensing mechanisms have been used to demonstrate the ability of carbon nanotubes to form nanoscale biosensors. The first sensing mechanism involves a CNT based conduction sensor in which the decrease in the current was observed when the specific biomolecule was bound. In the second mechanism Quartz Crystal Microbalance (QCM) was used to quantify the mass of the biomolecule bound on the sidewalls of the carbon nanotube. Both sensing mechanisms proved to be efficient and consistent. Immobilization of the biomolecules on the carbon nanotube surface was confirmed by Atomic Force Microscopy.

INTRODUCTION

Nanotechnology and Biotechnology are two emerging fields of science at the same size scale. Biological systems and structures such as found in DNA have served as new paradigms for the creation of many novel nanostructured materials. Carbon nanotube is an interesting material at the intersection of these two technologies. Although carbon nanotubes have many applications, one of the most important and promising applications which might find a significant place in the technologies related to these buckytubes is its biosensing ability [1]. The unique electronic properties of CNT in conjunction with the specific recognition properties of the incubated biomolecules would make CNTs as an ideal nanoscale biosensors. A close observation reveals that carbon nanotubes have a large surface area with all the carbon atoms on the surface. Hence, by a careful alteration of the surface chemistry of the carbon nanotube, they can be exploited for biosensing applications [2]. It has been recently demonstrated that individual semiconducting single wall carbon nanotubes can be used for the detection of glucose oxidase [3]. Controlled attachment of the glucose oxidase enzyme (GO_x) to the SWNT sidewall was achieved through a linking molecule which resulted in a clear change of the conductance of the sensing device. In the present work the electrical conductivity changes and the mass uptake of SWNT matrices on biomolecule incubation is reported.

EXPERIMENTAL DETAILS

Streptavidin from *Streptomyces avidinii* was purchased as a lyophilized powder from the Sigma-Aldrich Company. The Protein was dissolved in phosphate buffered saline (PBS, Sigma-Aldrich) and stored as aliquots at -20°C . Mouse monoclonal IgG was purchased from Bio Design International Inc. The antibody solution was diluted in PBS and stored frozen at -20°C until use.

Single wall carbon nanotubes (Corborex Inc.) were synthesized with nickel and yttrium as catalyst with 70% purity. The SWNTs have been dissolved in chloroform and then filtered through a $0.02\ \mu\text{m}$ pore size Anotop filters (Whatman). The filtered solution was then sonicated to derope the bundled CNTs. The CNTs were then casted on glass substrates. The glass substrates were cleaned before casting SWNT solution with isopropyl alcohol to remove any

contamination on the surface. The thickness of the film was controlled by the amount of casted solution and its concentration. The matrix of the SWNT was studied using Atomic Force Microscope (AFM; Thermomicroscopes Auto Probe CP Research machine) in non-contact mode. The thickness was measured using AFM by scanning along the edge of an artificially made scratch on the film.

Microelectrode contacts across the matrix were formed by silver thermal evaporation. The shadow masking technique was employed to form the gap between the electrodes. A tungsten wire (Sylvania) with a diameter of 60 μ m was wrapped around the glass substrate to mask the area of the CNT. After silver evaporation the tungsten wire was removed leaving the CNTs with the electrodes across them. A multimeter (Agilent-3458A) was employed to monitor the electrical changes of the CNT matrix which was interfaced with a computer. A constant voltage was applied between the electrodes and the conductance changes for various concentrations of the protein and antibody were recorded. Protein solutions were added to 10 μ l of PBS that had been placed on the sensor. PBS was employed as a buffer environment to distinguish changes in CNT matrix conductivity due to protein binding. Figure 1 shows a schematic representation of the SWNT based conduction sensor measurement set-up.

A Quartz Crystal Microbalance (Stanford Research Systems QCM100) with 5MHz AT-cut quartz crystals (gold coated) was used to quantitatively study the ability of the SWNTs to bind streptavidin and a mouse monoclonal IgG. SWNTs solutions were casted on the gold surface and then the crystal was baked at 50°C for two hours. The presence of SWNTs on the surface of the crystal was verified by Raman spectroscopy. The Raman spectrometer (Detection Limit Inc) was equipped with a laser source of 633nm wavelength and energy of 1.96eV.

RESULTS AND DISCUSSIONS

Figures 2 (a) and 2 (b) show the carbon nanotubes casted on silicon before and after filtration respectively. It is clearly evident that most of the undesired catalyst residue has been removed by filtration leaving the nanotubes. The thickness of the SWNT film was controlled by the concentration of the SWNT in the casting solution and the amount of solution used for casting.

Electrical characterization

Electrical characteristics of the sensor were studied using microelectrodes patterned by shadow masking technique as described above. Figure 3 shows the electrical characteristics of the SWNT matrix. The matrix demonstrated semiconducting behavior. This behavior is in agreement with the fact that a randomly selected SWNT sample contains approximately 70% semiconducting nanotubes while the rest are metallic [4]. Thus making the entire matrix semiconducting in nature.

Sensor responses

Five microliters of a solution of Streptavidin with different concentrations was added to the 10 μ l of PBS to result in final concentrations of 10nM, 1 μ M and 2 μ M of protein. Figure 4 shows the electrical response of the sensor to different protein concentrations. Point 1 indicates the instance at which 10 μ l of PBS was introduced between the electrodes and points 2, 3, and 4 indicate the

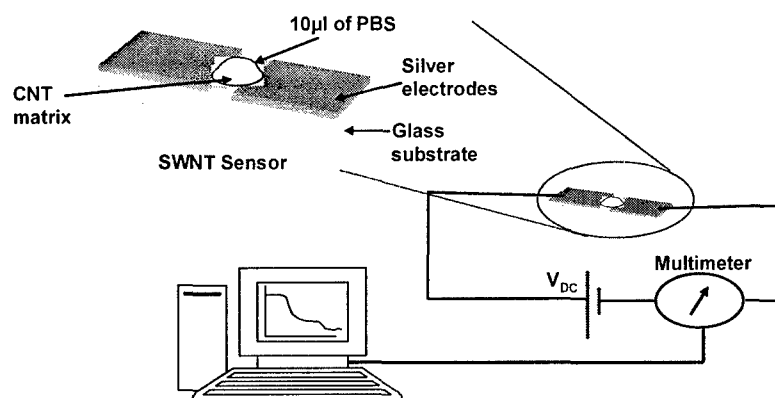


Figure 1. Schematic representation of the SWNT based conduction sensor measurement set-up.

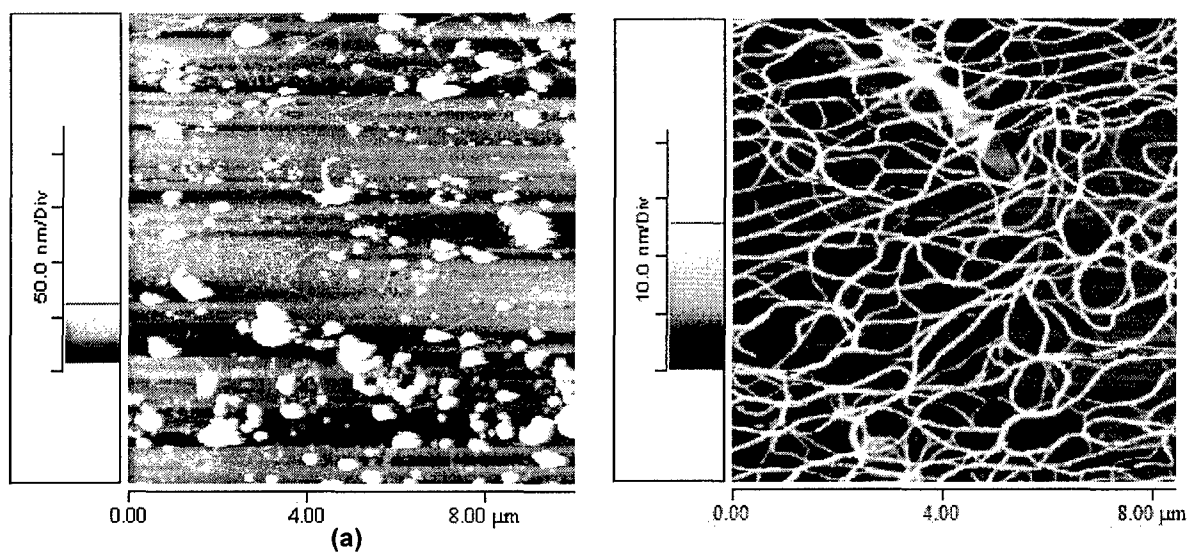


Figure 2. AFM images of SWNT before (a) and after filtration (b).

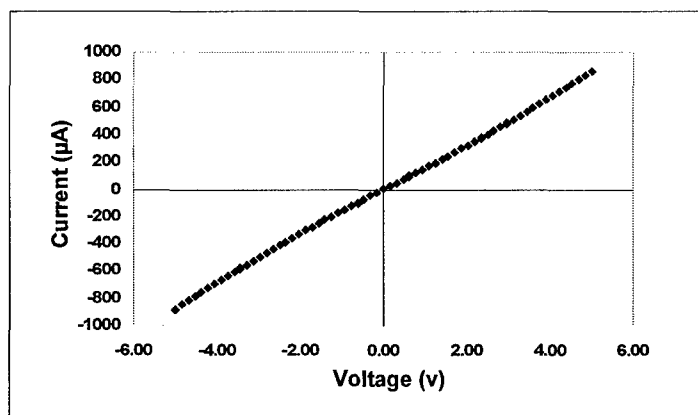


Figure 3. I-V characteristics of CNT matrix.

times when streptavidin solution was added to the PBS to form final concentration of the protein to be 10nM, 1 μ M, and 2 μ M respectively. It can be seen that there is no appreciable change in the current between the addition of PBS alone and the lowest concentration of streptavidin. Increasing the protein concentration to 1 μ M at Point 3 resulted in a decrease in the conductance of the SWNT matrix from 97.7 μ A to 60.3 μ A which corresponds to approximately 40% change in conductance. After the current stabilized the protein concentration was further increased to 2 μ M resulting in a further decrease in current. The change in conductance was 17.5% (60.3 μ A to 49.7 μ A) which is smaller compared to the initial change. These results indicate that most of the sites that are available for streptavidin to associate with the SWNT are already occupied for a concentration of 1 μ M.

Figure 5 (a) illustrates an AFM micrograph of the protein molecules bound on the surface of the substrate casted with SWNT and treated with 1 μ M of streptavidin and it can be seen that the streptavidin molecules mostly covered the SWNT network. Figure 5 (b) shows that SWNTs was decorated with streptavidin molecules when a concentration of 10nM the protein was used.

Figure 6 shows the sensor response to mouse monoclonal IgG. In this experiment following the introduction of PBS and 1 μ M of IgG the current decreased from 71.2 μ A to 45.4 μ A which is nearly a 36% change in conductance. With an increase in concentration of IgG to 2 μ M the current further decreased to 30 μ A which is a 30% change. This is consistent with Streptavidin behavior, which can be attributed to fewer active sites available for binding of IgG to CNT. The concentration of the biomolecule forms the “control” for the fine modulation of current between the electrodes. The spontaneous adsorption of both the biomolecules on the surface of carbon nanotube can be because of the hydrophobic interaction. The change in the conductance can be due to the charge transfer from biomolecule surface to the SWNT. SWNT is P-type semiconductor in nature and biomolecules are electron donors. Previous studies have shown that streptavidin is electrically neutral [5] at a pH between 6 and 7.2. However the surface of the protein molecule still consists of strong residual bases, which are responsible for charge transfer. Therefore electron transfer from biomolecule to the SWNT results in recombination of charge carriers which translates to conductivity reduction.

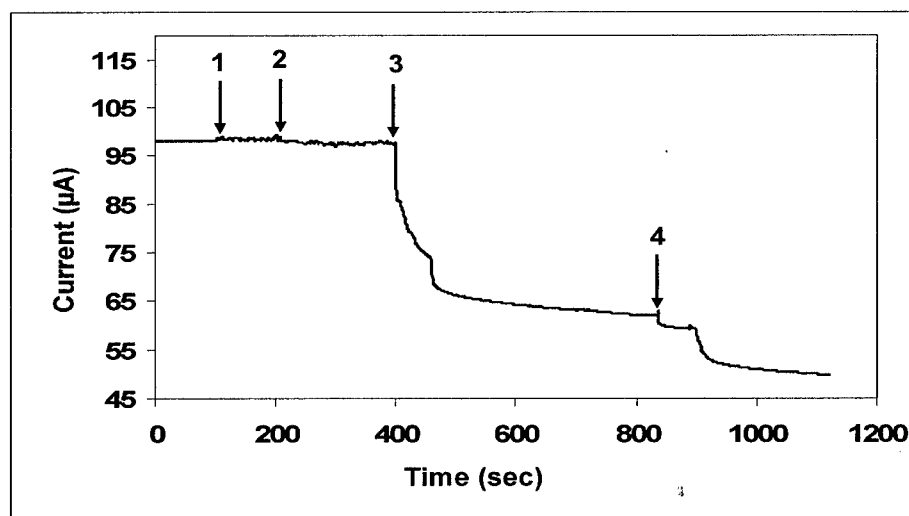


Figure 4. Electrical response of sensor for various concentrations of streptavidin.

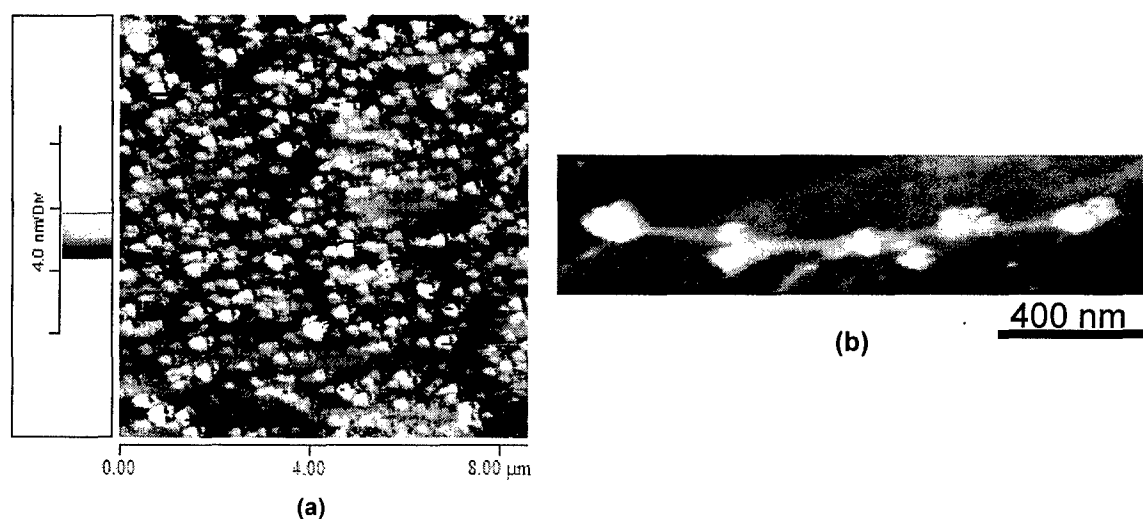


Figure 5. AFM micrograph completely covering SWNT matrix for a concentration of $1\mu\text{M}$ (a) and protein molecules decorating a rope of SWNT for a concentration of 10nM .

The quantitative study of mass uptake of SWNT network due to biomolecules immobilization was performed using QCM. In QCM a chemical interface on the surface of the sensor selectively adsorbs materials in the solvent to the surface of the sensing area. In our context the chemical interface is the SWNT matrix on the gold surface coated on quartz crystal.

Measurements of SWNT coated QCM crystals were performed by covering the chips with PBS before addition of the protein solutions. Figure 7 depicts the QCM response using streptavidin. For a concentration of $1\mu\text{M}$ of streptavidin a change of 120 Hz in resonant frequency was recorded. From the Sauerbrey equation the mass bound was calculated to be $1.538\mu\text{g}$. When the concentration on the chip was increased to $2\mu\text{M}$ the change in the frequency was found to be 26 Hz . This corresponds to a mass uptake of $0.33\mu\text{g}$. The lower frequency shift can be attributed to fewer active sites available for the protein molecules as described in the conductance based sensors. Similar results were observed for IgG with the frequency change being 248 Hz using a $2\mu\text{M}$ concentration.

CONCLUSIONS

This paper demonstrates two schemes of biomolecular detection using single wall carbon nanotubes network. The conductance based sensors exhibited a decrease in the current level due to the noncovalent binding of the biomolecules on the sidewall of the SWNTs. QCM experiments quantified the mass of the biomolecule bound on the SWNT matrix. Further investigation is underway to better understand the sensing mechanism and also improve the selectivity of the sensor through functionalization of carbon nanotubes.

ACKNOWLEDGEMENTS

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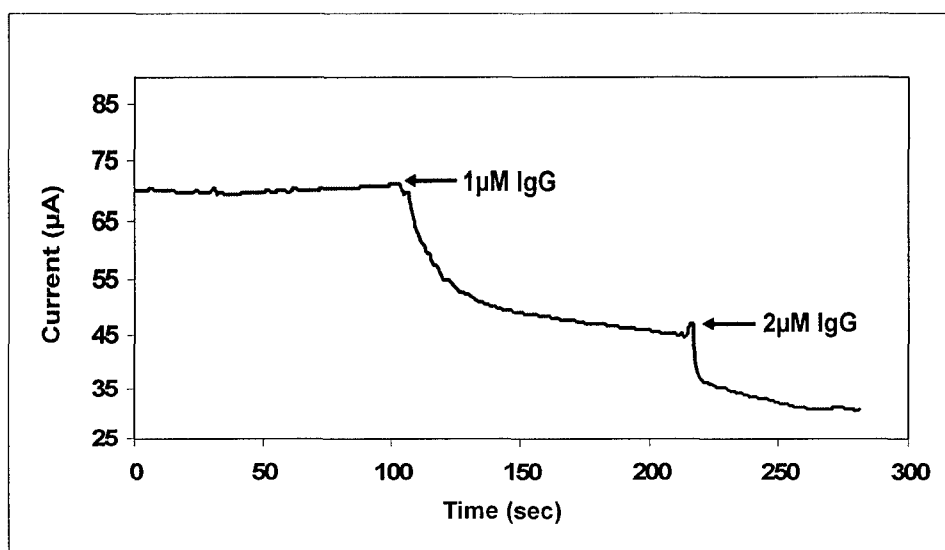


Figure 6. Electrical response of sensor for various concentrations of IgG.

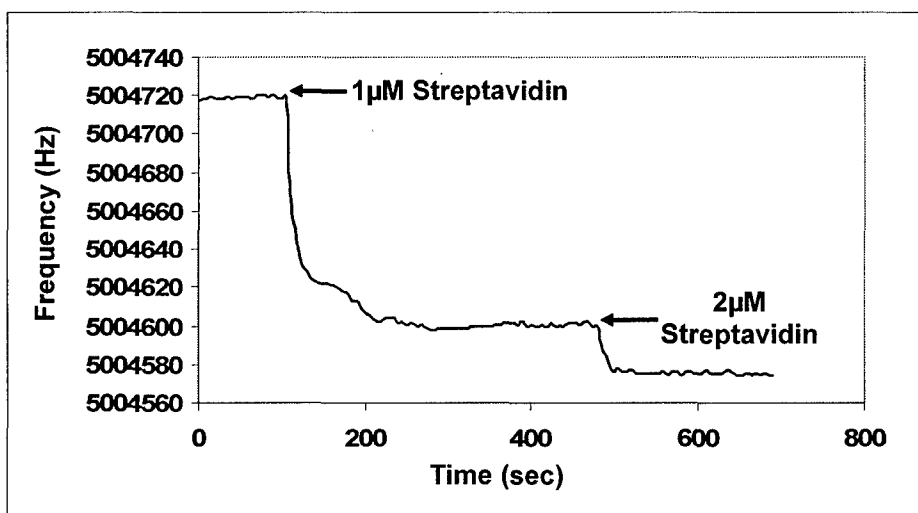


Figure 7. QCM response to streptavidin incubation.

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Carbon Nanotube Based Biosensors

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Abstract

In this paper we describe a single wall carbon nanotube (SWNT) based biological sensor for the detection of bio molecules like Streptavidin and IgG. SWNT have been employed for two types of sensing mechanisms. Firstly, the changes in the electrical conductance of the carbon nanotube matrix on non covalent binding of the biomolecules to the side walls of the carbon nanotube and secondly, quantification of mass uptake of the matrix on biomolecule incubation are presented. Both sensing mechanisms exhibited consistent and highly sensitive responses. Biomolecular immobilization on the carbon nanotube surface was monitored by Atomic Force Microscopy.

Keywords

Single walled Carbon nanotubes, Biomolecule, Atomic Force Microscopy.

INTRODUCTION

Single Wall Carbon nanotubes (SWNTs) can be realized as graphite sheets that have been rolled into seamless cylinders. Ever since Carbon nanotubes (CNTs) discovery by Iijima [1] in 1991, they have been treated as the most promising nanostructured materials. Carbon nanotubes exhibit both semi conducting and metallic behavior depending on their chirality [2]. This special property of nanotubes makes them the ideal choice for interconnects and also as active devices of nanoelectronics. CNTs have been used as chemical sensors for the detection of hazardous gasses such as NH_3 , and NO_2 [3]. The application of these quantum wires as biological sensors is a new facet which might find significant applications in the life sciences field [4] and it has been recently demonstrated that individual semiconducting single wall carbon nanotubes can be used for the detection of glucose oxidase [5].

In this work we have fabricated a simple yet efficient carbon nanotube conductance based sensor for the detection streptavidin and mouse monoclonal immunoglobulin G (IgG) antibody. In addition we employed Quartz Crystal Microbalance (QCM) to quantify the mass of the biomolecules bound on the surface of the nanotubes. QCM is a Thickness Shear Mode (TSM) acoustic wave device for detection of mass down to few nanograms.

EXPERIMENTAL DETAILS

Streptavidin from *Streptomyces avidinii* was purchased as a lyophilized powder from the Sigma-Aldrich Company. The Protein was dissolved in phosphate buffered saline (PBS, Sigma-Aldrich) and stored as aliquots at -20°C . Mouse monoclonal IgG was purchased from Bio Design International Inc. The antibody solution was resuspended in PBS and stored frozen at -20°C until use.

Single wall Carbon nanotubes (70% pure with nickel and yttrium as catalyst residue) were purchased from Corbolex Inc. The SWNTs have been dissolved in chloroform and then filtered through $0.02\ \mu\text{m}$ pore size Anatop filters (Whatman). The filtered solution was then sonicated to derope the bundled CNTs. The CNTs were then casted on glass substrates using a micropipette. The glass substrates were cleaned before casting SWNT solution with isopropyl alcohol to remove any contamination on the surface. The thickness of the film was controlled by the amount of casted solution and its concentration. The matrix of the SWNT was studied using Atomic Force Microscope (Thermomicroscopes Auto Probe CP Research machine) in non-contact mode. The thickness was measured using AFM by scanning along the edge of an artificially made scratch on the film.

Microelectrode contacts across the matrix were formed by thermal evaporation of silver. The shadow masking technique was employed to form the gap between the electrodes. A tungsten wire (Sylvania) with a diameter of $60\ \mu\text{m}$ was wrapped around the glass substrate to mask the area of the CNT. After silver evaporation the tungsten wire was removed leaving the CNTs with the electrodes across them.

Agilent multimeter (Agilent-3458A) was employed to monitor the electrical changes of the CNT matrix which was interfaced with a computer. A constant voltage was applied between the electrodes and the conductance changes for various concentrations of the protein and antibody were recorded. Protein solutions were added to $10\ \mu\text{l}$ of PBS that had been placed on the sensor. PBS was employed as a buffer environment to distinguish changes in CNT matrix conductivity due to protein binding (see Figure 1).

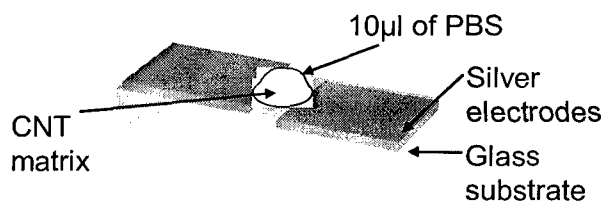


Figure 1. Schematic representation of the sensor.

Quartz Crystal Microbalance (Stanford Research Systems QCM100) with 5MHz AT-cut quartz crystals (gold coated) was used to quantitatively study the ability of the CNTs to bind streptavidin and mouse monoclonal IgG. CNT solution was casted on the gold surface and then the crystal was baked at 50°C for two hours. The CNT presence on the surface of the crystal was verified by Raman spectroscopy. The Raman spectrometer (Detection Limit Inc) was equipped with a laser source of 633nm wavelength and energy of 1.96eV.

RESULTS AND DISCUSSIONS

Purification of CNT

The CNTs were dissolved in chloroform (Sigma-Aldrich) and then casted on silicon substrates. However AFM imaging of the samples revealed that there was a high content of catalyst residue. Filtration of the CNT solution using antop filters resulted in transparent solution. Figure 2 shows the photograph of the CNT solution before and after filtration. It is known that the carbon nanotubes have a tendency to form into parallel bundles resulting to triangular lattice because of the inter-molecular van der Waals forces between the nanotubes [6]. To overcome the inter-molecular forces the filtered CNT solution was sonicated for 1 hour.

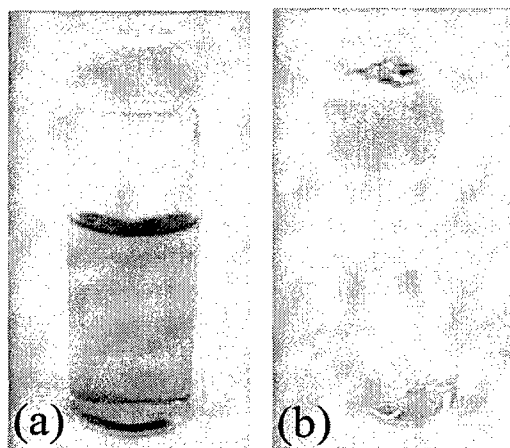


Figure 2: Photographs showing the solution of CNT (a) before and (b) after filtration

Figure 3 shows uniformly distributed casted CNT matrix on the silicon substrate. It can be observed that most of the undesired catalyst residue has been filtered out leaving behind the nanotubes.

The thickness of the CNT film was controlled by the amount of solution casted and the concentration of the solution. Figure 4 shows the micrograph of the CNT film and its height profile showing that the film thickness was approximately 20nm.

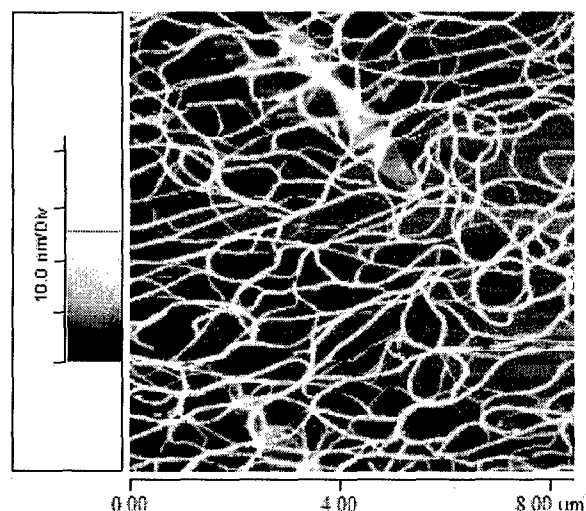


Figure 3. AFM micrograph of CNT matrix on silicon substrate.

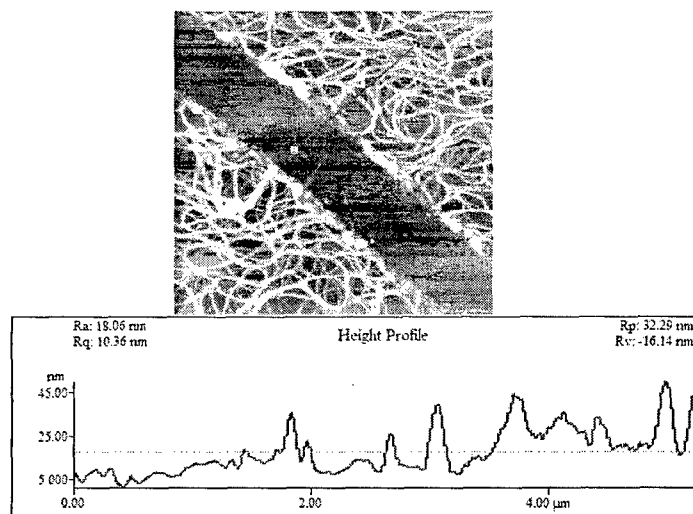


Figure 4. AFM micrograph of CNT matrix showing scratch and height profile.

Electrical characterization

Electrical characteristics of the sensor were studied using microelectrodes patterned by shadow masking technique as described above. Figure 5 shows the electrical characteris-

tics of the CNT matrix. The matrix demonstrated semiconducting behavior. This behavior is in agreement with the fact that a randomly selected CNT sample contains approximately 70% semiconducting nanotubes while the rest are metallic [2]. This makes the entire matrix to be semiconducting in nature.

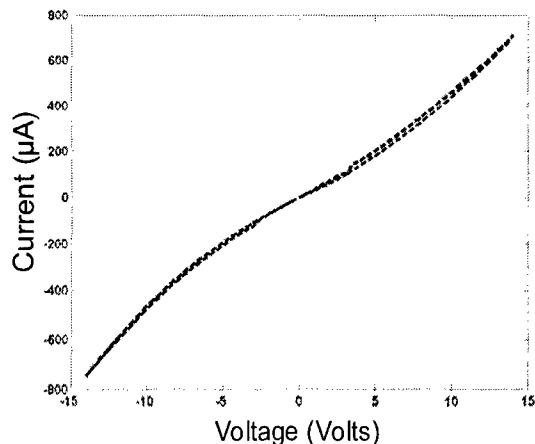


Figure 5. I-V characteristics of CNT matrix.

Sensor responses

Five microliters of a solution of Streptavidin with different concentrations was added to the 10 μ l of PBS to result in 10nM, 1 μ M and 2 μ M of protein. Figure 6 shows the electrical response of the sensor to different protein concentrations. Point 1 indicates the instance at which 10 μ l of PBS was introduced between the electrodes and point 2 is the time at which 5 μ l streptavidin solution was added to the PBS making the final concentration of the protein to be 10nM. It can be seen that there was no appreciable change in the current. The protein concentration was increased to 1 μ M at Point 3 and a decrease in the conductance of the CNT matrix was observed. The current decreased from 97.7 μ A to 60.3 μ A which corresponds to approximately a 40% change in conductance. After the current stabilized the protein concentration was further increased to 2 μ M and a further decrease in current was recorded. The change in conductance was 17.5% (60.3 μ A to 49.7 μ A) which is smaller compared to the initial change.

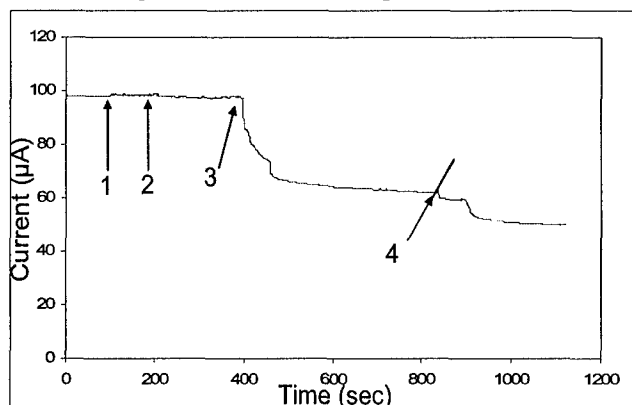


Figure 6. Electrical response of sensor for various concentrations of streptavidin.

The smaller change in the conductance can be attributed to less number of active sites available for the protein molecules to bind to CNT.

Figure 7 is an AFM micrograph of the CNT and protein molecules. From this Figure it can be seen that protein molecules were bound on the sidewalls of the tube and bundles of CNT were decorated with streptavidin molecules.

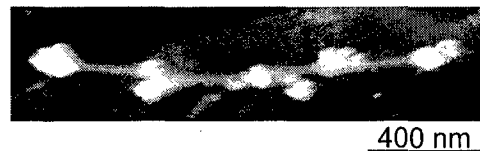


Figure 7. AFM micrograph showing the protein molecules decorating a rope of CNT.

Figure 8 shows the sensor response to the mouse monoclonal IgG. Following introduction of PBS and 1 μ M of IgG the current decreased from 71.2 μ A to 45.4 μ A which is nearly a 36% change in conductance. With increase of concentration to 2 μ M the current further decreased to 30 μ A which is a 30% change. This is consistent with Streptavidin behavior which can be attributed to less number of active sites available for binding of IgG to CNT.

The concentration of the biomolecule forms the "control" for the fine modulation of current between the electrodes. The change in the conductance can be explained in a simple way. It is known from previous studies that streptavidin is electrically neutral at a pH between 6 and 7.2. However the surface of the protein molecule still consists of strong residual bases. These bases are responsible for charge transfer.

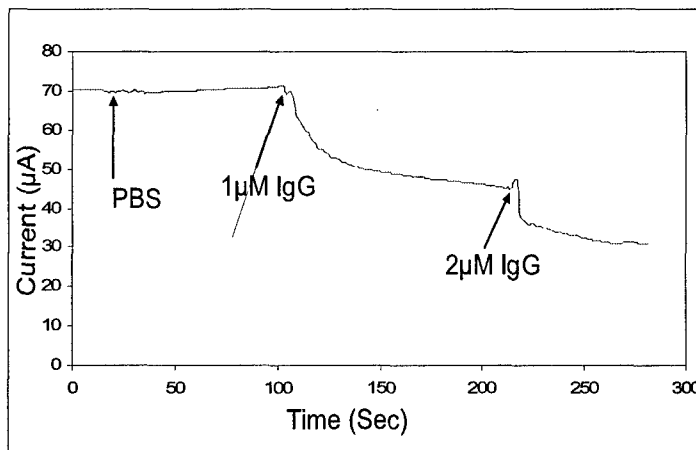


Figure 8. Electrical response of sensor for various concentrations of IgG.

The quantitative study of mass uptake of CNT network due to biomolecules immobilization was performed using QCM. In QCM a chemical interface on the surface of the sensor selectively adsorbs materials in the solvent to the surface of the sensing area. In our context the chemical

interface is the CNT matrix on the gold surface coated on the QCM crystal. The CNT matrix on the gold surface was characterized by Raman spectroscopy [7]. Figure 9 shows the Raman spectrum obtained from the CNT film on the gold surface of the quartz crystal. It can be seen that the Raman spectrum has characteristic 'G' band and 'D' band peaks which arise due to the in-plane Raman-active mode and disorder of the CNT respectively [7].

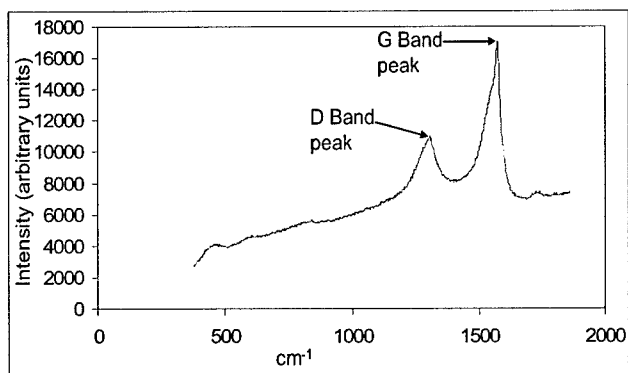


Figure 9. Raman spectrum of CNT on gold surface of QCM showing the characteristic 'G' band and 'D' band peaks.

Measurements of CNT coated QCM crystals were performed by covering the chips with PBS before addition of the protein solutions. Figure 10 depicts the QCM response using streptavidin. For a concentration of $1\mu\text{M}$ of streptavidin a change of 120 Hz in resonant frequency was recorded. From the Sauerbrey equation the mass bound was calculated to be $1.538\mu\text{g}$. when the concentration on the chip was increased to $2\mu\text{M}$ the change in the frequency was found to be 26Hz. This corresponds to a mass uptake of $0.33\mu\text{g}$. The lower frequency shift can be attributed to fewer active sites available for the protein molecules as described in the conductance based sensors. Similar results were observed for IgG with the frequency change being 248Hz using a $2\mu\text{M}$ concentration.

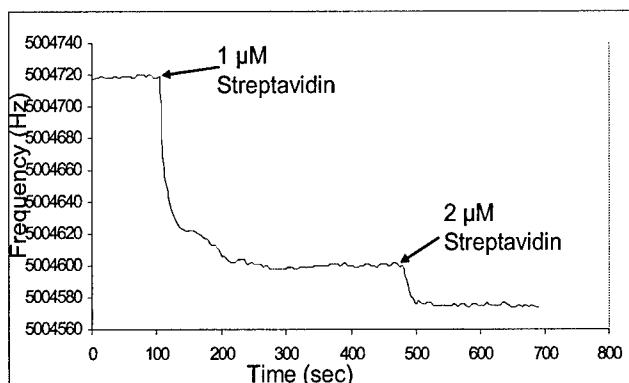


Figure10. QCM response to streptavidin incubation.

CONCLUSIONS

We have demonstrated a simple and efficient method for purification of carbon nanotubes for the fabrication of conductometric biosensor. Two schemes of biomolecular sensing using carbon nanotubes have been demonstrated. The conductance based sensors exhibited a decrease in the current level due to the noncovalent binding of the biomolecules on the sidewall of the CNTs. QCM experiments quantified the mass of the biomolecule bound on the CNT matrix. Further investigation is underway to better understand the sensing mechanism and also improve the selectivity of the sensor by functionalizing carbon nanotubes.

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Sensitivity Enhancement of a QCM Biosensor using Polymer Treatment

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Abstract--Quartz Crystal Microbalances (QCMs) have been widely used for detection of various chemical and biological species in liquid media. We report an improved binding of Protein A and IgG molecules on QCM biosensors by modifying the gold surface of the quartz crystal with a 35nm polystyrene film followed by an acidic treatment. An appreciable increase in the frequency shift was observed when the polystyrene film was used as an interfacial layer. Protein A and IgG immobilizations on quartz crystals with the polystyrene film represented a 65% increase and a 40% increase respectively when compared to immobilization done directly onto the crystals. Complementary Atomic Force Microscopy (AFM) studies revealed a significant decrease in the RMS roughness of the substrate from 98.4 nm to 1.75 nm when coated with polystyrene. This increased surface smoothness resulted in higher biomolecular coverage on the surface of the sensor causing higher frequency shifts.

I. INTRODUCTION

Existing immunoassay techniques for biomolecular detection such as fluoroimmunoassay (FIA), enzyme linked immunoassay (ELISA) and radioimmunoassay (RIA) are complex and time consuming as they require specific labels and reagents [1, 2]. Biosensors with rapid and highly sensitive detection capabilities for various biomolecules are therefore in great demand in the field of life sciences. Acoustic wave sensors have been widely used for the specific detection of various chemical and biological molecules in liquid media. The chemical interface on the surface of the sensor selectively adsorbs

materials in the solvent to the surface of the sensing area. Due to this adsorption, the physical and chemical properties of the surface change thus altering the phase and amplitude of the acoustic and electromagnetic fields on the surface. For biological sensors, binding of a substance onto the resonating membrane surface causes a decrease in the acoustic wave velocity, which is related to the resonant frequency of the device. The changes in the acoustic and electromagnetic properties of the chip can be directly proportional to the changes in mass.

Quartz Crystal Microbalances have been extensively used for gravimetric immunoassays of human serum albumin (HSA) [3], monoclonal mouse IgG [2], and human IgG [1]. The simple relationship relating the change in frequency (Δf) to the change in mass (Δm) enables QCM to be widely used in sensing applications. A shift in the resonant frequency of the QCM can be attributed to the mass bound on the sensor membrane as per the Sauerbrey equation

$$\Delta f = \frac{-2f_0^2 \Delta m}{A\sqrt{\rho_q \mu_q}} \quad (1)$$

where f_0 is the fundamental resonant frequency, A is the piezoelectrically active area defined by the two gold electrodes, ρ_q is the density of quartz (2.648 g cm^{-3}) and μ_q is the shear modulus ($2.947 \times 10^{11} \text{ dyn cm}^{-2}$). Equation (1) is based on an assumption that the mass has been rigidly attached to the crystal and has negligible thickness as compared to the crystal as a whole [1].

II. EXPERIMENTAL DETAILS

1. Materials

Polystyrene, 3-Aminopropyl triethoxysilane (3-APTES), glutaraldehyde, acetone, glycine and sodium chloride were purchased from Sigma-Aldrich Chemical Company. Protein A (Sigma-Aldrich) and mouse monoclonal IgG antibody (BioDesign International Inc) were used throughout these studies. Protein A was resuspended in phosphate buffered saline (PBS; Sigma-Aldrich) at a concentration of 500 μ g/ml.

2. Experimental Procedures

For promoting the immobilization of Protein A and to provide the necessary amine groups on the gold surface the protocol of Muramatsu et al. (1987) was followed [1]. The crystal was cleaned with Piranha solution (3 parts of H₂SO₄ in 1 part of 30 % H₂O₂) to remove any organic contamination from the surface of the crystal. Enough Piranha solution was employed to cover the gold surface of the chip and allowed to incubate at room temperature for two minutes before rinsing with milli-Q water. This procedure was repeated twice. The chip was subsequently blown dried in a stream of nitrogen gas. A self-assembled monolayer (SAM) was created using a 5% solution of 3-APTES in acetone. After 1 hour at room temperature the chip was placed in a 5% (v/v diluted in milli-Q water) glutaraldehyde solution for 3 hours to enhance the cross linking between the chip and the Protein A. The crystal was then covered with 20 μ l of a solution of Protein A (0.5 mg/ml diluted in PBS; pH7.4). After 1 hour the solution was removed and the crystal was subjected to several wash-dry cycles with milli-Q water until the QCM crystal reached its steady resonant frequency. The chip was then covered with 0.1 M glycine dissolved in PBS for 1 hour to block any sites not bound to Protein A on the glutaraldehyde modified chip. The chip was then rinsed with 0.1M glycine-HCl buffer (pH 2.4) to wash off any excess proteins or glycine before being thoroughly rinsed with milli-Q water. 20 μ l of the mouse monoclonal IgG solution was then incubated on the chip for 1 hour

followed by rinsing with 0.5M NaCl to remove any non-specifically adsorbed antibody. For the experiments in which binding was performed on a polymer film, polystyrene solution (7% w/v in chloroform) was spin coated onto the chip at a speed of 1000 rpm and then treated with 50 % (v/v) HNO₃ in concentrated H₂SO₄ for 1 hour [8]. The substrate was then modified with 3-APTES followed by glutaraldehyde as described above. The procedure was repeated for different concentrations of IgG on crystals with and without the polymer film and the frequency shifts were monitored.

3. Characterization tools and methods

A Quartz Crystal Microbalance (Stanford Research Systems QCM100) with 5MHz AT-cut quartz crystals (gold coated) was used to quantitatively study the ability to bind Protein A and mouse monoclonal IgG to the chip. The gold surface, which forms the active area for immobilization was 1.37cm² and the mass sensitivity of the crystal was 0.057 Hz/ng/cm². Frequency was monitored using a Stanford Research System Universal Time Interval Counter (Model No. SR620).

Qualitative studies were made using AFM (Autoprobe CP Research machine) in non-contact mode. For AFM studies silicon substrates were used with the same modification techniques as those described above for the QCM chips. The AFM tips used for imaging were silicon with an approximate radius of curvature of 20nm. Biomolecular imaging was performed in non-contact mode. The AFM images were analyzed using image-processing software (IP 2.1) to calculate the RMS roughness value.

III. RESULTS AND DISCUSSIONS

The AFM studies revealed that the commercially purchased quartz crystals had a RMS surface roughness of 98.4 nm. To determine if decreasing the RMS surface roughness would increase the efficiency of biomolecule binding and hence

improvement in antibody immobilization, the quartz crystals were coated with an ultra thin polymer film. Coating the surface with polystyrene resulted in a significant decrease in RMS surface roughness to 1.75 nm.

However, polystyrene films are hydrophobic in nature causing the biomolecules to denature and hence loose their activity. To avoid denaturation of the biomolecules, the polymer film functional groups such as amino and hydroxyl groups can be chemically added. This helps the biomolecules retain their activity as immobilization now takes place through the hydrophilic arms of the polymer film. To increase the hydrophilicity of the surface which would increase the ability to add the functional groups, the chips were subjected to an acidic treatment followed by aqueous silanization.

Figure 1 shows the schematic representation of the acidic treatment and the APTES modification of polystyrene. The acid treatment provides NO_2 groups and the APTES modification improves the

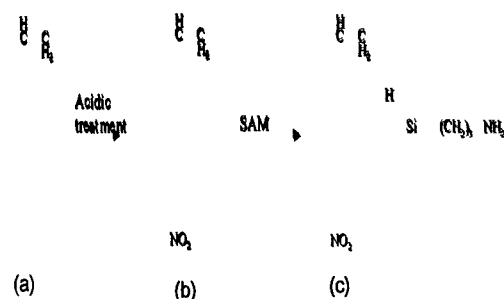


Figure 1. Polystyrene film when treated with acid and 3-APTES; (a) Polystyrene film (b) formation of the NO_2 groups by acidic treatment (c) formation of the amine groups by silanization.

hydrophilicity of the surface by providing the required amine groups.

Figure 2 shows the AFM image of IgG immobilized on polystyrene coated surface. It can be seen that there is a uniform coverage of the antibody molecules of approximately 10 nm in size on the substrate. The AFM imaging performed two

hours after the biomolecules immobilization revealed that the molecules still retain their

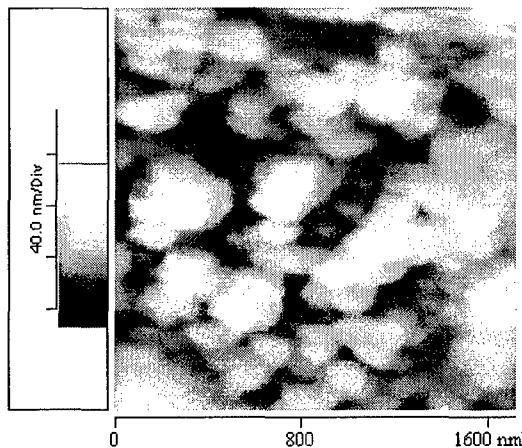


Figure 2. IgG molecules immobilized on the polystyrene surface showing the characteristic heart shape indicating that they still are not denatured.

The biomolecule immobilization on gold and polystyrene coated gold surfaces was then quantitatively studied with QCM. Figure 3 shows the QCM frequency response to Protein A immobilization without the polystyrene film. Protein A, which has a particularly high affinity for the F_c portion of IgG, was immobilized first on the chips to prevent the random immobilization of the antibodies, and therefore maximizing the ability of the chip immobilized antibodies to bind to antigens [2].

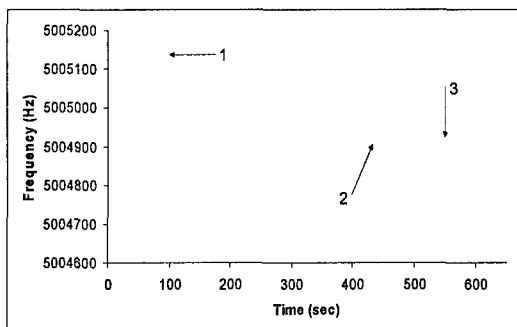


Figure 3. QCM frequency response for Protein A immobilization on polystyrene.

In Figure 3 point 1 indicates the time of addition of the Protein A containing solution to the chip. Point 2 shows when the crystal was subjected to several wash-dry cycles and point 3 represents the new resonance frequency of the crystal when Protein A was specifically bound on the surface. The frequency shift due to this direct binding (the difference in frequency between point 1 and point 3) was 220 Hz. From the Sauerbrey equation, this frequency shift corresponds to a binding of 2.8 μg of protein. 160 $\mu\text{g}/\text{ml}$ solution of IgG was added to the Protein A modified chip. The chip was rinsed with 0.5M NaCl solution to remove the nonspecifically adsorbed IgG. The final frequency shift for the antibody immobilization was found to be 282 Hz which represents a mass of 3.61 μg .

Immobilization on polystyrene coated surfaces was compared to the immobilization performed without polystyrene. Figure 4 shows the QCM response to Protein A immobilization performed on polystyrene surface.

The registered frequency shift from point 1 to point 3 was 364 Hz which corresponds to a mass change of 4.66 μg . This represents a 65% increase when compared to the QCM chips that were not coated with the polymer film. Similar results were obtained for the binding of IgG. The QCM response for the immobilization of IgG (160 $\mu\text{g}/\text{ml}$) on the

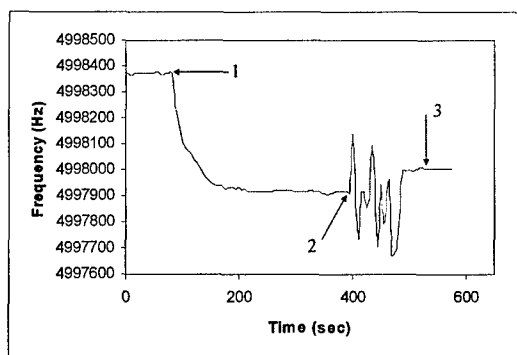


Figure 4. QCM frequency response for Protein A immobilization on polystyrene.

polystyrene surface showed a frequency shift of 391 Hz corresponding to a mass uptake of 5.01 μg . This represents a 40% increase when compared to chips that had not been modified with polystyrene.

IV. CONCLUSIONS

We have demonstrated a simple aqueous silanization technique to improve the ability of QCM gold chips to bind to protein A and subsequently IgG. Higher frequency shifts were observed for immobilizations carried on polymer-coated surfaces. Protein A resulted in a shift of 364 Hz on the polystyrene modified surface as opposed to the 220 Hz shift of frequency for direct immobilization. Subsequent IgG immobilizations also showed similar trend with the frequency shifts being 391 and 282 Hz for immobilizations with and without the polymer film respectively. AFM imaging also revealed uniform biomolecule coverage on polymer coated surfaces.

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PIEZOELECTRIC BIOSENSOR FOR PROSTATE CANCER DETECTION

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Existing antibody detection techniques such as fluoroimmunoassay, radioimmunoassays and enzymeimmunoassays require specific labels and markers for detection, which makes them very complex and time consuming. To develop an assay for serum antigens that is simpler and less labor intensive we have used an acoustic wave biosensor for the detection of antibodies against free prostate specific antigen (fPSA). A Quartz Crystal Microbalance (QCM100) was used to measure the levels of immobilized antibody. It consisted of AT-cut Quartz crystal having a resonant frequency of 5 MHz and a mass sensitivity of 0.057 Hz/ng/cm². Any change in the mass on the membrane results in a change in the frequency of operation (Δf), which is related to the mass immobilized (Δm) as per the Sauerbrey equation

$$\Delta f = \frac{-2f_0^2 \Delta m}{A \sqrt{\rho_q \mu_q}}$$

To enhance the association of glutaraldehyde with the surface of the microbalance chip, a self-assembled monolayer (SAM) was formed by treating the substrate with aminopropyl triethoxysilane (3-APTES). To confirm the formation of the SAM Atomic Force Microscopy (AFM) studies were performed. AFM studies revealed a significant increase in the roughness of the surface of the chip from 0.44nm to 15.8nm confirming the formation of SAM. As antibodies added to these chips may bind in random orientations which could result in a high degree of variability in the ability of immobilized antibodies to bind to the appropriate ligand, we immobilized Protein A (500µg/ml) on the treated gold surface. AFM demonstrated a uniform coverage of the surface with molecules of approximately 10nm in size, which correlated well with previously published reports on the size of Protein A. Frequency shifts were approximately 220Hz, which indicated that 2.82µg of protein A had been immobilized. Any remaining active sites were blocked by glycine and different concentrations of antibody against fPSA were reacted with the chip. Frequency shifts were studied for different concentrations of antibody. QCM measurements for antibody concentration of 80µg/ml revealed a frequency shift of 90Hz corresponding to a mass of 1.153µg. At a concentration of 120µg/ml a frequency shift of 120 Hz was observed which corresponds to a mass of 1.538µg. These results indicate the feasibility of using quartz microbalances for the measurement of PSA.

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