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Microgravimetric immunosensor for direct detection of aerosolized influenza A virus particles

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Abstract

The development and characterization of a quartz crystal microbalance (QCM) sensor for the direct detection of aerosolized influenza A virions is reported. Self-assembled monolayers (SAMs) of mercaptoundecanoic acid (MUA) are formed on QCM gold electrodes to provide a surface amenable for the immobilization of anti-influenza A antibodies using NHS/EDC coupling chemistry. The surface-bound antibody provides a selective and specific sensing interface for the capture of influenza virions. A nebulizer is used to create aerosolized samples and is directly connected to a chamber housing the antibody-modified crystal (“immunochip”). Upon exposure to the aerosolized virus, the interaction between the antibody and virus leads to a dampening of the oscillation frequency of the quartz crystal. The magnitude of frequency change is directly related to virus concentration. Control experiments using aerosols from chicken egg allantoic fluid and an anti-murine antibody based immunosensor confirm that the observed signal originates from specific viral binding on the chip surface. Step-by-step surface modification of MUA assembly, antibody attachment, and antibody–virus interaction are characterized by atomic force microscopy (AFM) imaging analysis. Using the $S/N=3$ principle, the limit of detection is estimated to be 4 virus particles/mL. The high sensitivity and real-time sensing scheme presented here can play an important role in the public health arena by offering a new analytical tool for identifying bio-contaminated areas and assisting in timely patient diagnosis.

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Keywords: Quartz crystal microbalance; Biosensor; Influenza virus; Immunosensor; Self-assembled monolayer

1. Introduction

Human infection by the influenza virus is a significant public health concern throughout the world. According to the World Health Organization, there are an estimated three to five million severe cases and between 250,000 and 800,000 deaths each year [1]. Infection occurs through inhalation of contaminated droplets or direct contact with contaminated surfaces [2,3]. The ease of transmission and the growing commerce of worldwide human travel readily enable viral epidemics and pandemics. The negative impact on society is not only the morbidity and mortality, but also the direct effect on the world economy.

Influenza is an enveloped single stranded RNA virus, with two major glycoproteins (haemagglutinin, HA and neuraminidase, NA) that evenly project from the surface. These transmembrane proteins determine the antigenicity of the

influenza A and B viruses. Influenza A has one of sixteen different HA proteins (H1–H16), and one of nine different NA proteins (N1–N9) on its surface. To successfully provide immunity, the development of vaccines focuses on targeting these surface antigens. Mutation of these sites (termed “antigenic drift”) results in a compromise of immunity. Antigenic *shift* is another genomic occurrence in which there is an exchange of HA between viruses. This occurs when a cell is infected with two different influenza viruses of the same subtype and genetic exchange occurs during replication, from H3 to H6, for instance. These phenomena explain seasonal epidemics and necessitate annual production of new vaccines, which target the evolving cell surface antigens. Even though many infections are prevented through vaccination, it should not be relied on as the only source of protection. This was exemplified in 2004, when one of the world’s largest manufacturers of the influenza virus vaccine had to recall their bacteria-contaminated product.

In recent years, significant progress has been made in the treatment of influenza infection. Because of a short therapeutic window, medications to treat flu are most efficacious when

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delivered early in the course of the disease. Therefore, current anti-flu regimens positively impact morbidity and mortality if infection is immediately detected and diagnosed [4]. However, many microbiological methods that determine influenza infection take several days or weeks to obtain results. New methods to identify the virus in infected individuals or contaminated environments include reverse transcriptase polymerase chain reaction (RT-PCR), immunofluorescence (IF), and enzyme-linked immunosorbant assay (ELISA). These methodologies often suffer from long analysis times, complicated procedures, non-quantitative results, and high costs [5]. The need for highly trained individuals and large expensive instruments also render these analysis methods unappealing [6]. Most significantly, these techniques are not *in situ* methods of analysis, and require environmental and patient sample collection to be performed separately and independently from laboratory-executed identification, detection and analysis [7,8].

The complexities of environmental viral identification and detection, patient diagnosis and pharmaceutical intervention have promoted the need for the development of new methods for influenza measurement and quantification. Biosensors are emerging as the method of choice in many applications. The intrinsic characteristics of biosensors such as high sensitivity, reliability, and portability, as well as, low cost of operation, fabrication, and maintenance make them attractive alternatives to conventional analysis methods. Several sensors for influenza virus detection in the aqueous phase are commercially available, offering results in 30 min with 70% sensitivity and 90% specificity for the viral antigens [5]. Despite the obvious need and a wide range of possible applications, analytical instrumentation capable of fast, sensitive and direct measurements of airborne influenza has not yet been fully explored. Biological aerosol mass spectrometry (BAMS) has only recently emerged for detection of microorganisms with no reagent consumption [9]. Fluorescence aerodynamic particle sizing [10], multiwavelength UV fluorescence spectroscopy [8] and semi-nested RT-PCR [11] have also proven effective for gas phase bacterial detection and identification. The Reyes group reported a bio-aerosol fluorescence sensor for detection of *B. subtilis* (a substitute for *B. anthracis*) and *Erwinia herbicola* (a substitute for *Y. Pestis*) [11]. The instrument detects and identifies bioaerosols by using two photomultiplier tubes (PMT), specially tuned for tryptophan and NADH. They achieved detection limits of 5–10 particles/L, and were able to discriminate between threatening biologicals in the air and “background” aerosols such as vehicle emissions. Unfortunately, the aforementioned techniques usually possess technological set-backs that include time consuming procedures, and expensive reagents and instruments.

In recent years, acoustic-based sensors such as the quartz crystal microbalance have attracted considerable interest for the development of novel sensors. The QCM exploits the piezoelectric properties of a quartz crystal wafer. An inverse piezoelectric effect occurs when an electric field is applied across electrodes placed on both sides of the crystal which leads to a physical deformation of the crystal [12]. Perturbation of the fundamental frequency of the crystal is attributed to a change of mass on the

modified electrode surface. The frequency and mass change on a QCM crystal surface is expressed by the Sauerbrey equation [13]:

$$\Delta f = -C_f(f_0)^2 \left(\frac{\Delta m}{A} \right) \quad (1)$$

where Δf is the frequency change, C_f the Sauerbrey constant, f_0 the fundamental resonant frequency of the crystal, Δm the change in mass, and A is the sensing area of the crystal. Sensitivity of the QCM can be in the ng/cm^2 range, and relies on various factors including method of surface modification, rigidity of the sensing interface and the thickness and size of the quartz wafer. The requirement of tagging the analyte with a fluorescent or radioactive label is not necessary for this surface sensitive technique which makes QCM sensors an attractive analytical tool. Piezoelectric sensors for DNA, bacterial toxins, bacterial spores and many other biological molecules have been reported [14–16]. Several gravimetric sensors for the indirect detection of viruses identify viral DNA, proteins released by infected cells, or antiviral antibodies [17–21]. Sato et al. detect influenza using a QCM with membrane bound monoganglioside GM_3 as the capture agent [22]. Although proven effective, these QCM sensors detect analyte in liquid media, and have not been shown effective for analysis of bioaerosols. Despite the fact that QCM sensors for the gas phase are more sensitive than their liquid phase counterparts, gas phase sensors based on QCM technology are mostly limited to small molecule detection [23–25]. But more recently, a piezoresistive microcantilever sensor for the detection of vaccinia virus in solution and in aerosols was reported [26]. Bashir and co-workers use an array of microcantilevers to measure the mass of a single vaccinia virus particle [27]. A piezoelectric immunosensor for the detection of the SARS-associated coronavirus in sputum has also been reported [28], showing good reproducibility, stability and a linear working range of 0.6–4 $\mu\text{g}/\text{mL}$.

In this work, we report a piezoelectric immunosensor for the detection of aerosolized influenza A (H3N2) virions. Influenza is a logical target for an aerosol-based sensor because virus-contaminated airborne droplets remain infectious for long periods of time. Polyclonal anti-influenza A (H3N2) antibodies are used as the capture molecules. To construct the sensing interface, a SAM of mercaptoundecanoic acid (MUA) is formed on the gold electrode of the quartz crystal, and EDC/NHS coupling chemistry is employed for covalent linkage of the anti-influenza antibody (IgG) to the SAM surface. Aqueous solutions of influenza virus are aerosolized using a nebulizer and the aerosolized particles are enclosed within a chamber in which the immunochip is placed. In this report, we also investigate the sensing properties of the chip, in particular the response time and sensitivity. In addition, a series of control experiments confirm that the sensor signal is generated from the specific binding between flu particles and the immobilized antibodies. Recent reports show that atomic force microscopy (AFM) provides useful in the imaging of biomolecules such as proteins and antibodies [29–32]. Imag-

ing of the step-by-step modification of the sensor surface is also presented. This QCM methodology for real-time aerosolized influenza detection is simple, rapid, and inexpensive; opening new doors for the development novel sensing technologies for viruses.

2. Experimental

2.1. Chemicals and biologicals

Influenza A virus (VR-544, H3N2) was acquired from American Type Culture Collection. Goat anti-influenza A, H3N2 polyclonal unconjugated antibody was from Fitzgerald Industries International. Anti-murine polyclonal unconjugated antibody was from purchased from Sigma. Mercaptoundecanoic acid ($\text{HOOC}(\text{CH}_2)_{10}\text{SH}$, MUA) was from Aldrich. *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) were from Acros. Gold Shield Chemical Co. provided the absolute ethanol. All chemicals were used as received, with no further purification performed. Stock virus samples were stored at -80°C in 100 μL of PBS buffer with an initial concentration of 64 HAU, and diluted to desirable concentrations for aerosol analysis. Millipore ultra-pure water was used in the preparation of all buffer solutions, for dialysis of samples used in nebulization experiments and for the dilution of biological aqueous samples. All solutions used in mass detection were dialyzed in ultra-pure water overnight at 4°C using cellulose ester (CE) dialysis membranes (MWCO = 100,000).

2.2. Quartz crystal microbalance immunochip fabrication

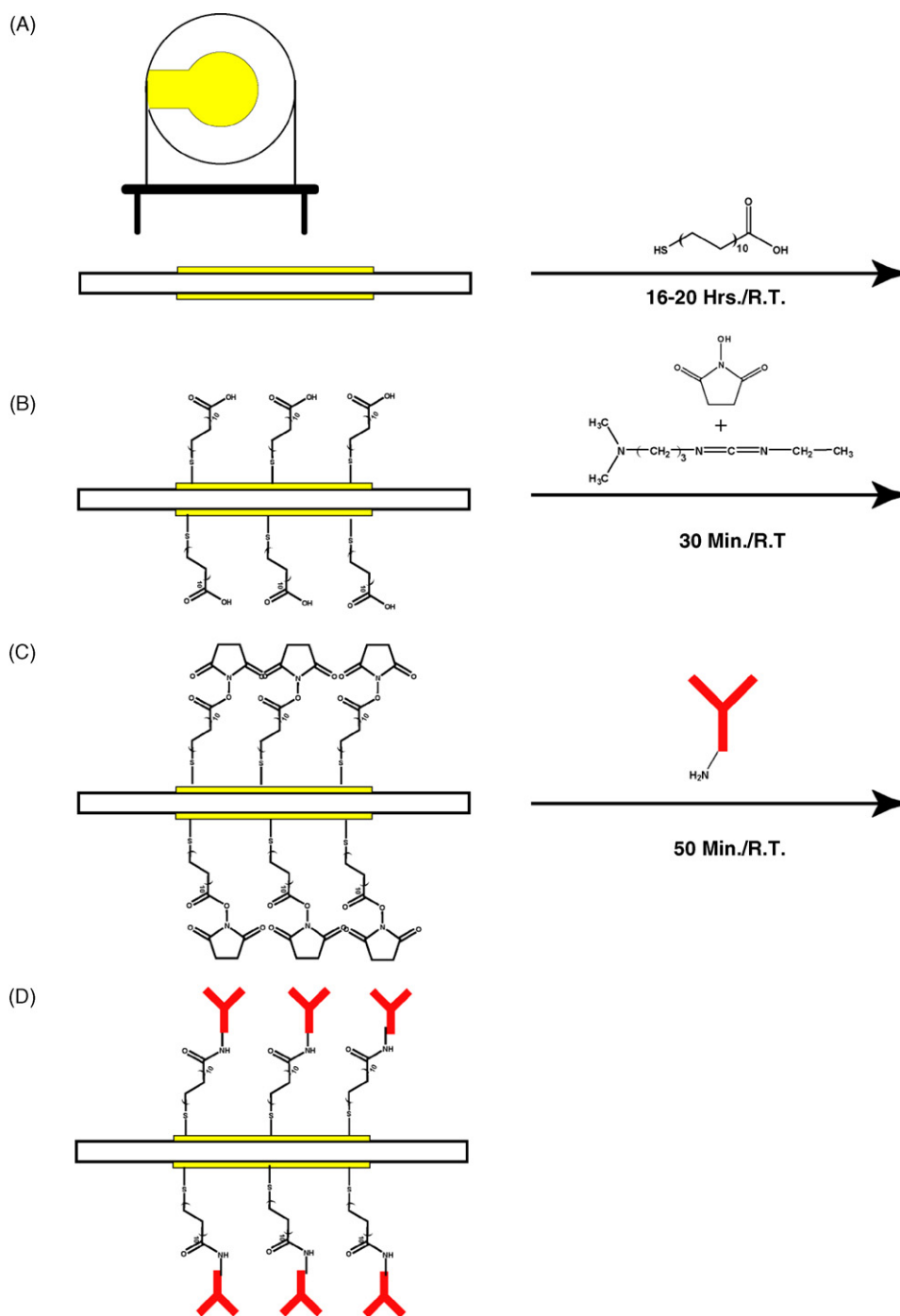
The fabrication procedure of the virus sensing interface is represented in Scheme 1. Before surface modification, crystals were rinsed with absolute ethanol and then cleaned with piranha solution [3:1 (v:v) conc. H_2SO_4 :30% H_2O_2 . Caution! Piranha reacts violently with organics]. The crystals were then washed with copious amounts of ethanol and water and blown dry by a stream of pure nitrogen. The first step in surface modification was formation of the MUA SAM. Crystals were immersed in a 10 mM MUA ethanolic solution overnight (16–20 h) at room temperature. Next, they were removed from the thiol solution and rinsed with absolute ethanol and dried under nitrogen. The crystals were then placed in a solution of EDC/NHS for 30 min at room temperature to form the NHS ester compound. After formation of the ester, the crystal was washed with water, and dried with nitrogen. Finally, the surface modification was completed by placing the ester modified crystal in a solution of antibodies (either anti-influenza or anti-murine) for 50 min. Antibody side-chain lysine residues formed a covalent amide bond between the surface-bound MUA-NHS ester and the antibody. After modification, the crystals were rinsed with PBS buffer and ultra-pure water, and dried gently with a stream of nitrogen. Antibody-modified crystals (“immunochips”) were then immediately connected to the oscillator and placed into the nebulization chamber for aerosol analysis.

2.3. Frequency measurements of aerosol samples

Frequency measurements of the QCM were made using an Agilent/Hewlett-Packard (HP 53131A) frequency counter attached to a personal computer. Frequency data were collected using the software provided by the frequency counter manufacturer. The 10 MHz oscillator unit and AT cut 10.0 MHz crystals were purchased from International Crystal Manufacturing. The crystals had a 100 Å thick Ti adhesion layer and a 1000 Å thick gold layer. An Omron Micro Air nebulizer was used to generate aerosol particles. This device utilizes a ceramic mesh plate and low frequency vibration to create low velocity aerosol. After surface modification, the crystal was placed into a 50 mL nebulization chamber. Virus dialysate was diluted with ultra-pure water to the desired concentration and this solution was placed in the nebulizer. The chamber provided isolation and confinement of aerosolized particles for the detection of airborne virus particles. Once the crystal gave a stable frequency reading, the nebulizer was turned on for 3 s and then turned off (this will be referred to as a “pulse”). The minimum nebulization rate was 0.25 mL/min, and particle mass median aerodynamic diameter (MMAD) was 5 μm . Live influenza was deactivated by UV radiation in a UVP CL-1000 ultraviolet crosslinker. As a further safety precaution, all nebulization experiments were carried out in a Level 2 Biosafety cabinet.

2.4. Atomic force microscopy surface analysis

Modified and unmodified QCM gold surfaces (2 cm \times 2 cm) were placed on an AFM (Novascan ESPM 3D) sample holder. Images were taken on separate QCM chips after each step of fabrication. Piranha solution was used to clean a new unmodified chip and washed with water and ethanol before imaging. A separate clean chip was then immersed in thiol solution to create the SAM modified surface, rinsed exhaustively with ethanol and water, dried under nitrogen and immediately placed on the sample holder and then imaged. Antibodies were immobilized on a separate MUA-NHS ester modified chip, rinsed with buffer and ultra-pure water, dried and immediately imaged. Finally, an immunochip was placed in the nebulization chamber and exposed to 3 pulses of virus aerosol. Before imaging, the chip was exhaustively rinsed with buffer and ultra-pure water and then dried with nitrogen. The AFM was placed inside a vibration isolation chamber to minimize external vibrations and interference. The AFM tip (Silicon tip Novascan NSC35 with spring constant of 7.5 N/m, resonance frequency of 210 kHz and a nominal tip radius of 10 nm) was carefully positioned over the region of interest (typically at the center of the sample). Non-contact mode (intermittent contact mode) was used to scan all samples. A scan resolution was set at 400 lines \times 400 lines and a scan rate of 1 Hz. The scanned area was an 80 μm \times 80 μm region. Best resolution was obtained when the set point = -0.47 V and amplitude = 0.005 V . The final images were flattened horizontally and vertically using edge fit, and truncated between 0 and 500 nm to obtain the best contrast of surface roughness.



Scheme 1. Step-by-step procedure for fabrication of QCM immunosensor chip; (A) MUA SAM formation, (B) NHS ester formation, (C) antibody immobilization, (D) QCM immunosensor chip.

3. Results and discussion

3.1. Response of the QCM immunosensor to aerosolized influenza virus

A nebulizer was used to generate aerosolized influenza particles of consistent size to simulate airborne particles from a sneeze or cough. Fig. 1 shows the frequency response of a crystal modified with influenza specific antibodies when exposed to a nebulized aqueous solution of influenza. Virus solutions were

dialyzed to prevent erroneous frequency readings from salt formation on the sensor surface upon water evaporation. The virus solution concentration was approximately 1.5 haemagglutinating units (HAU) per mL dialyzed allantoic fluid. After the first pulse ($t = 35\text{--}38$ s), an overall net frequency change of 1694 Hz was recorded ($t = 114$ s). Frequency shifts were calculated from the point at which the first pulse begins minus the point where the signal stabilizes (also the beginning of the second pulse). Binding curves were of similar shape to those previously reported [31,33]. However, the frequency shifts for similar concentrations

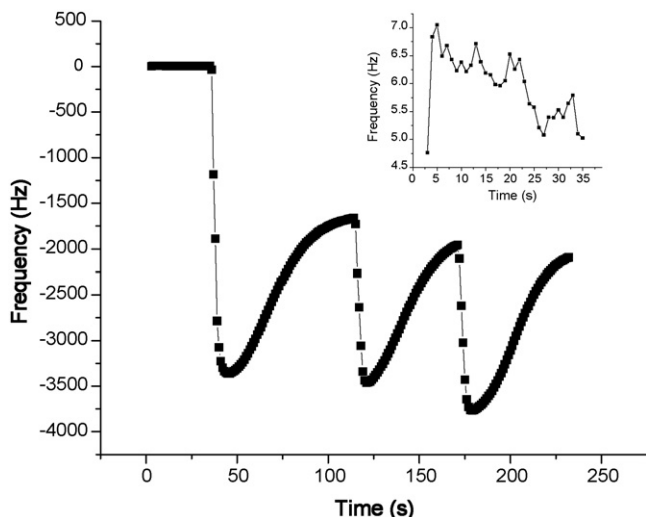


Fig. 1. A sensorgram obtained when an anti-influenza modified immunochip is exposed to 3 pulses of a nebulized 1.5 HAU/mL influenza virus solution. The inset is the noise within the frequency signal from $t=0$ to 35 s.

of analyte observed in this study were much larger, by one or two orders of magnitude. Clearly the immunosensor gave an immediate and distinctive response, eliminating the need for sample incubation that other systems require [21,34]. An observed successive decrease in sequential pulses suggests a reduction in available surface binding sites for antibody–antigen interactions.

In order to verify that the frequency changes observed in Fig. 1 were a direct result of antigen–antibody interactions, several control experiments were performed. The first control was the analysis of the sensor chip response to aerosolized ultra-pure H_2O , which is shown in Fig. 2. There was an initial rapid decrease in frequency of 1089 Hz ($t=19$ s) upon nebulized water entering the chamber. However, the frequency returned to the baseline after each nebulization, showing that there was no net perturbation in sensor signal from the water aerosol. The second control experiment analyzed the effect of allantoic fluid on the frequency response, as the virions used for these experi-

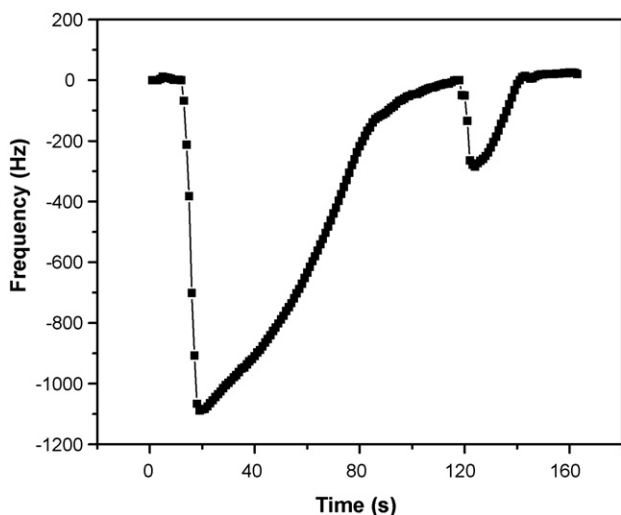


Fig. 2. The effect of nebulized ultra-pure water on the oscillation frequency of the immunosensor chip modified with anti-influenza IgG.

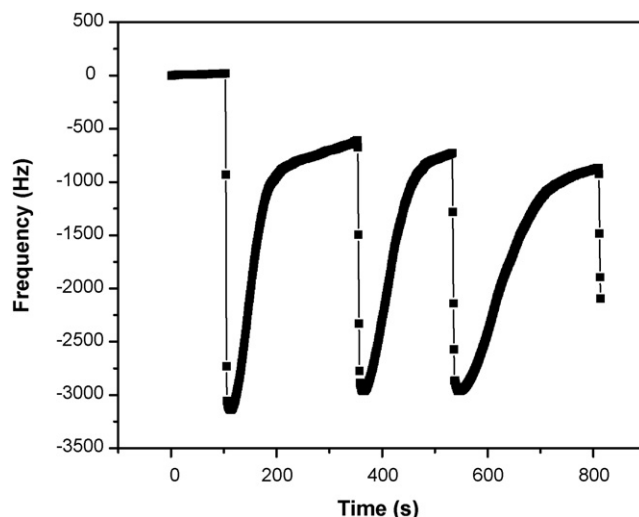


Fig. 3. The frequency response of the anti-influenza IgG immunosensor chip when exposed to concentrated nebulized allantoic fluid.

ments were cultured in chicken eggs. Allantoic fluid is a complex matrix comprised of lipids, carbohydrates and proteins, which is one of three extensions of a developing bird embryo. The response of the sensor exposed to concentrated nebulized allantoic fluid is represented in Fig. 3. There was a noticeable net change in the crystal frequency of 696 Hz ($t=103$ s) after the first nebulization, probably caused by non-specific binding of proteins or lipids present in the fluid. Subsequent pulses produced a net change in resonant frequency much smaller than the first pulse ($\Delta f_2 = 51$ Hz), associated to saturation of non-specific binding sites. We would like to point out that the large change in frequency was initiated by a concentrated solution of allantoic fluid. Also, because the antibodies used here are polyclonal, there are antibodies in the stock solution that respond to the biomolecules present in allantoic fluid. The concentration of the biomolecules found in the virus samples was dramatically less than in a pure solution of allantoic fluid. This would be especially true for virus solutions in the low concentration

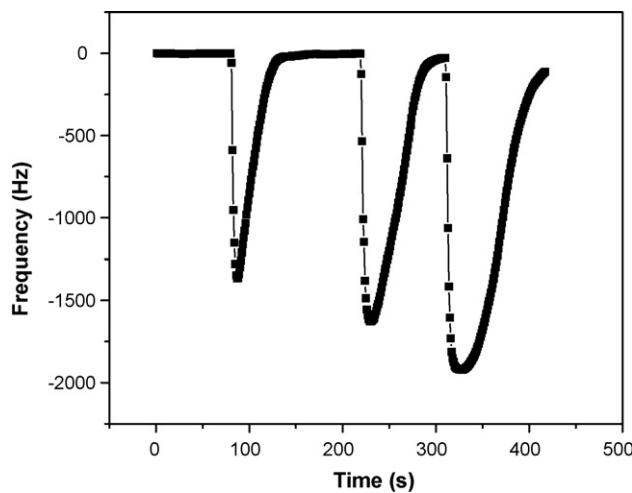


Fig. 4. Control experiment showing the frequency change of an anti-murine IgG modified sensor chip when aerosolized influenza is introduced into the nebulization chamber.

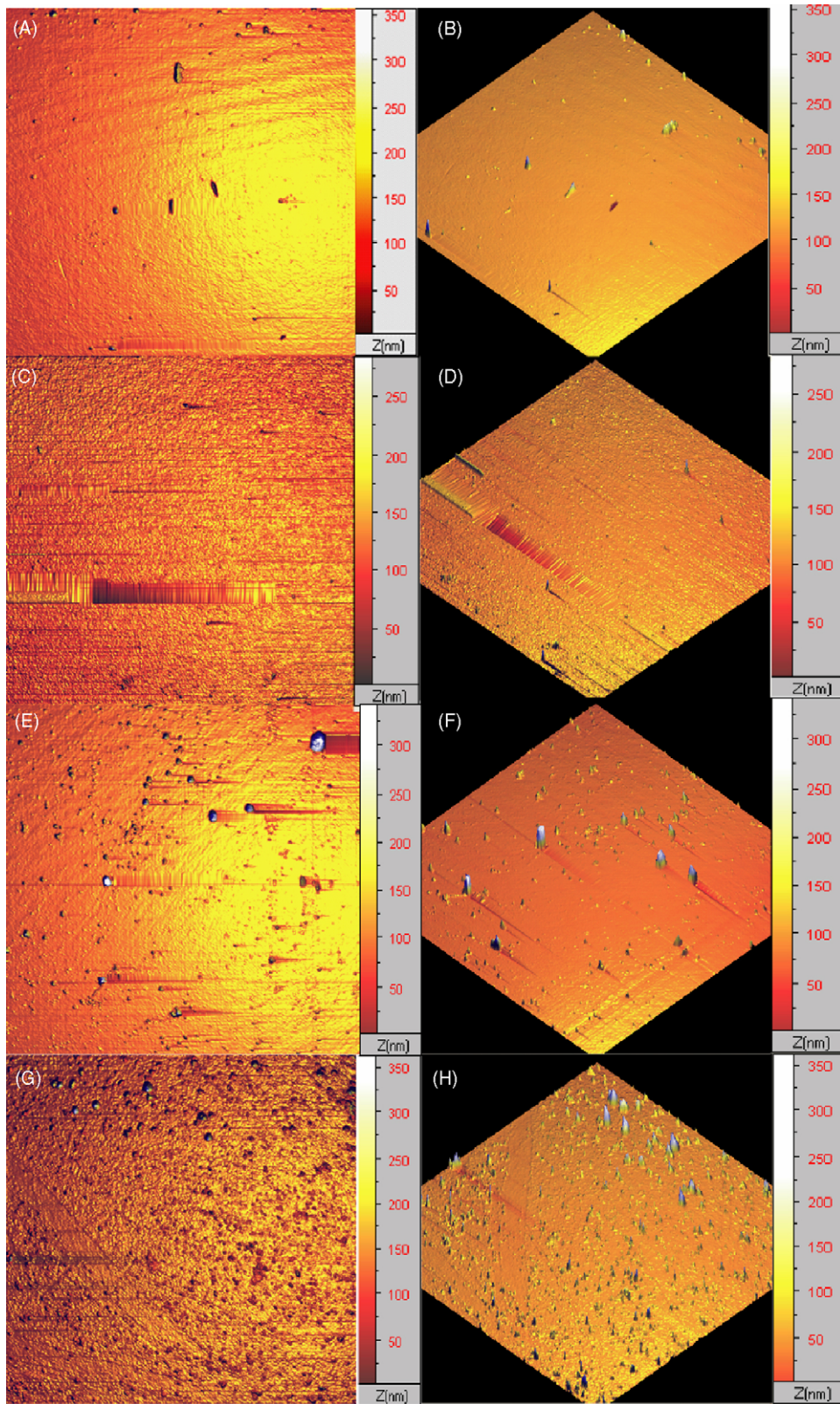


Fig. 5. X–Y plane and isometric view of AFM images of QCM gold substrate surfaces; (A and B) clean gold surface, (C and D) after MUA assembly, (E and F) after anti-influenza IgG immobilization, (G and H) after exposure to aerosolized influenza virus.

regime. However, it is impossible to quantify the concentration of biomolecules in the concentrated allantoic fluid sample and likewise, the concentration of those same molecules in the virus samples. In comparison to these results, the net decrease in resonant frequency in Fig. 1 can be attributed to specific antigen–antibody interactions. In addition, the shape of the sensor response to the virus sample was very different when compared to experiments in which there was no specific binding (Figs. 2 and 3). Specific binding occurred within 78 s after nebulization begins, which is clearly shown in Fig. 1.

3.2. Anti-murine IgG immunochip for virus control experiments

To further confirm that the signature binding response in Fig. 1 was induced by virus–antibody interaction, additional control experiments were performed using anti-murine antibodies to functionalize the sensor chips. The response of the anti-murine immunochip to the nebulized influenza particles is shown in Fig. 4. The first pulse resulted in a 1365 Hz decrease in frequency, and as expected the frequency nearly returned to the base line 139 s later ($\Delta f=0.3$ Hz). The shape of the resulting binding curve is similar to that of the anti-influenza IgG chip for water, clearly indicating that there was no significant binding of the virus on the anti-murine IgG surface. This result provided additional evidence that confirms specific binding occurred between anti-influenza IgG and the influenza particle, which led to the dampening of oscillation frequency shown in Fig. 1. As discussed above, the antibody for influenza used in this work is of the polyclonal unconjugated variety. Thus, exposure of the immunochip to aerosolized allantoic fluid results in the change in frequency because there are antibodies responsive to the biomolecules present in the matrix.

3.3. Topographic analysis of the immunosensor using AFM

We carried out further characterization of the immunochip functionalized with anti-influenza IgG by atomic force microscopy. AFM images were acquired for several steps of sensor fabrication and after exposure to aerosolized virus. Fig. 5A and B are the X–Y plane view and the isometric view, respectively, of a clean gold QCM crystal before any surface modification. The topography of the clean electrode was relatively smooth, especially when compared to the other imaged surfaces. After the formation of the MUA SAM, the AFM-imaged surfaces revealed slight roughness (Fig. 5C and D). Images taken after covalent linkage of anti-influenza IgG are shown in Fig. 5E and F. It is clearly visible from the images that a further change in surface roughness was a result of IgG attachment. The differences between the MUA SAM and the immobilized IgG surface demonstrate the close packed nature of the monolayer and a high density of antibodies on the surface. Size of antibodies is consistent with change in image. It should be noted that the coverage of antibodies may not be necessarily uniform on the $80\ \mu\text{m} \times 80\ \mu\text{m}$ imaging area. Fig. 5E and F also show several areas with large features (>60 nm), possibly due to antibody clusters. The final AFM images, Fig. 5G and H, were

acquired after exposure to 3 pulses of influenza aerosol. The sensor surface texture was obviously affected by the exposure to the aerosolized virus. While both Fig. 5F and H exhibit large features due to dust particles or aggregates, the density of features with a height of 80–120 nm (the expected size of the influenza virus [5]) increases by at least a factor of 10 when comparing Fig. 5F–H. Thus, exposure of the surface to aerosolized virus led to a much higher number of features whose size is consistent with virus particles bound to the surface. While the AFM images cannot provide chemically specific information about the objects on the surface, the changes in surface morphology at every step in the sensor fabrication process are consistent with our hypothesized sequence of SAM and antibody attachment, followed by virus detection. These observations provide additional evidence that each step in the sensor fabrication process resulted in a topographical change of the substrate surface.

3.4. Calibration curve for the QCM immunochip

A linear relationship for sensor signal with regard to virus solution concentration is shown in Fig. 6. The sensor shows a linear response over three orders of magnitude. Each 3 sec pulse delivered approximately $10\ \mu\text{L}$ of solution into the chamber, based on calculations derived from the specifications of the nebulizer. A 2 Hz fluctuation in the frequency response (inset Fig. 1), considered as noise, was used to estimate the lowest detection limit. By estimating that $1\ \text{HAU} = 10^6$ virus particles [5], a $\Delta f = 151$ Hz for a solution of 0.02 HAU/mL concentration of virus particles yields a detection limit of 4 particles/mL of space, derived from the $S/N=3$ principle. Lamb reported that the mass of a single influenza particle with a 100 nm diameter is 7.4×10^{-9} g, based on the sum total of nucleic acids, proteins, lipids that compose one influenza virion [35]. From this data, the limit of detection is calculated to be 29.6 ng/mL.

The sensitivity of the QCM method demonstrated in this work was excellent for a first generation sensor of this type. Compared to liquid media experiments previously reported, the technique

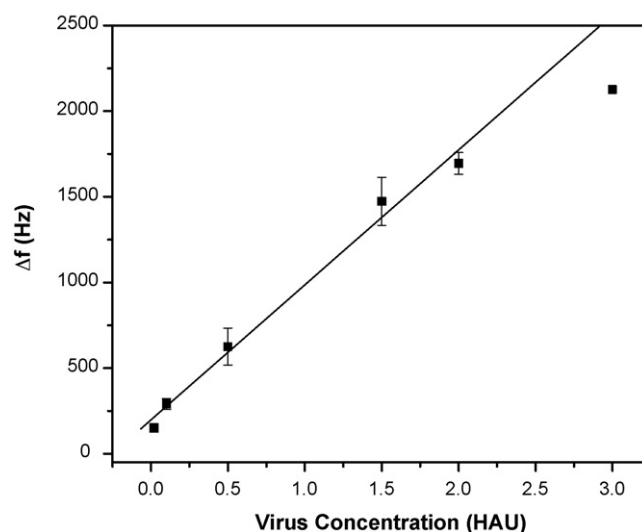


Fig. 6. The calibration curve of the anti-influenza IgG chip, correlating frequency shift to the solution virus particle concentration.

presented here has an improved sensitivity. The *Salmonella* sensor by Fung and Wong had a reported ~ 35 Hz shift for a sample containing 10^5 cells, and a detection limit of 1.7×10^2 cells/mL [34]. Perhaps that both sides of the crystal are modified in this report for sensing aerosolized samples accounts for the sensitivity enhancement.

4. Conclusions

This work demonstrates the development of a QCM-based immunosensor for direct detection of aerosolized influenza A virus particles. The sensor responded to the specific target and gave a real-time signal, which was very distinguishable from those observed in the control experiments. The limit of detection for this system is 4 particles/mL, or 29.6 ng/mL, better than the values reported previously by other sensors and by ELISA. The sensor demonstrates a linear response between 0.02 HAU and 3 HAU. There was no frequency deviation due to liquid loading, liquid pressure on the crystal surface or liquid pump noise as found in fluid based experiments. Pre-culture, separation and incubation steps were not needed between the virus sample collection and detection/identification for this analysis technique. The ease of fabrication, high sensitivity and selectivity, fast analysis time, and the elimination of radioactive or fluorescent labels represent the tremendous advantages achieved by this biosensor.

AFM imaging demonstrated the formation of the MUA SAM, immobilization of the anti-influenza IgG, and the capture of aerosolized influenza by an anti-influenza IgG that was surface-bound to the QCM substrate. Compared to other similar sensor systems, this method has the benefit of detecting the actual viral particle, not a byproduct of a viral infection – and most importantly – detection of the virus particle in an aerosolized sample. Miniaturization and automation of such a QCM system would allow for real-time and continuous monitoring of air conditioning and air circulation within schools, airports, federal buildings and hospitals. The simple and uncomplicated response would allow for individuals with very little training to interpret a positive or negative signal—a desirable feature of any biosensor system. A clinical sensor for non-invasive diagnosis of respiratory tract infection by analysis of patient breath samples is also foreseeable. A myriad of selective viral sensors based on this sensing platform would be possible by investigating the use of various receptors or antibodies.

However, there are a few aspects that may significantly affect the sensor performance. It is well known that the SAM method for antibody immobilization can be problematic because antibodies may not always be immobilized in an orientation that renders them available for antibody–antigen interactions. This would lead to variability among sensor chips. Another cause for chip-to-chip variation is the denaturing or deactivation of surface-bound antibodies [36], especially in the gas phase. Finally, the fact that the sensor was detecting particles in a large chamber (50 mL) as opposed to a small chamber (on the order of tens of μL) commonly used in liquid phase experiments should also be considered when evaluating the performance of this sensing system. Diffusion of aerosolized particles is a more significant variable as compared to that of particles flowing through

a liquid flow cell system, and must be treated through a different set of empirical methods. Nevertheless, this work is the first successful demonstration of a QCM-based immunosensor for real-time sensing of aerosolized influenza virus particles.

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