EXPLOSIVE TRACE DETECTION WITH FBAR-BASED SENSOR

Anderson Lin[1], Hongyu Yu[1], Michael S. Waters[2], Eun Sok Kim[1], and Steven D. Goodman[2]
[1]Department of Electrical Engineering-Electrophysics,
[2]Department of Molecular and Computational Biology
University of Southern California, Los Angeles, California, USA

ABSTRACT

This paper describes explosive trace detection through mass sensing with film bulk acoustic resonator (FBAR) coated with antibodies. Mass sensing for a specific explosive is done with the use of antibody made specifically for the targeted explosive. Antibodies were immobilized on the backside of FBAR mass sensors using an antibody immobilization protocol. When the specific explosive is exposed to the sensor, it binds to the antibody on the sensor, causing a shift in the resonant frequency of the FBAR sensor. This sensing technique has been shown to selectively detect vapor traces of TNT (Trinitrotoluene, a common explosive) and RDX (Cyclotrimethylenetrinitramine which has one of the lowest vapor pressures among various explosives) without any pre-concentrator.

1. INTRODUCTION

In the fight against terrorism, the ability to detect traces of explosives in their vapor phase is essential for the safety of all citizens. One method for such detection is using HPLC (high-performance liquid chromatography), which gives very few false alarms, but is laborious and expensive. In searching for alternatives, we would like something fast, cheap and portable. One possible candidate would be using resonant mass sensors with high mass sensitivity such as FBAR.

In an attempt to minimize the possible false alarms that come with detection by mass sensing, we utilized the antibody-antigen binding concept in our mass sensing method, exploiting the known high binding specificity between an antibody and its targeted antigen. In order to accomplish this, we would need to apply a layer of antibody on our mass sensing devices, while making the antibody available for binding with antigen, or explosive vapor, in air. This process is quite challenging, because the binding process must occur in the vapor phase, instead of the liquid phase that is usually done in most antibody-antigen essays.

Since the binding is to occur in the vapor phase, there have been concerns that binding sites on an antibody may lose its prescribed structure in the absence of an aqueous environment [1], thus there may be a need for a hydration layer for the antibodies so that the antibodies will be able to function. However, there has been a report of vapor phase detection using antibody without a hydration layer utilizing surface plasmon resonance spectroscopy [2]. If this is correct, we would not have to worry about how the explosive vapor will be able to gain access to the antibodies without getting trapped in the hydration layer. This paper describes our preliminary data that support a vapor-phase mass sensor based on antigen-antibody binding without any hydration layer. Also shown are the experimental demonstrations of trace detection of TNT and RDX vapors without any pre-concentrator that have been possible due to the extremely high sensitivity of the FBAR-based mass sensing.

2. DESIGN AND FABRICATION

FBAR Fabrication

FBAR fabrication was done by first depositing low stress LPCVD silicon nitride on a (100) silicon wafer. Silicon nitirde on the backside of the wafer was patterned, and the silicon substrate was etched in KOH solution to produce the supporting silicon nitride diaphragm on the frontside. To produce the sandwich structure of FBAR, bottom electrodes were first deposited in an e-beam metal evaporator, with Cr used as an adhesive layer for Au. After patterning of the bottom electrodes, piezoelectric ZnO film was sputter-deposited and patterned. Top electrodes were then formed using lift-off technique after depositions of Au/Cr in an e-beam metal evaporator. Then, a gold layer was deposited on the backside of the silicon nitride diaphragm for antibody immobilization for selective mass sensing. The fabricated FBARs have thicknesses of 0.08 µm/0.66 µm/0.08 µm/0.8 µm/0.1 µm for Au/ZnO/Au/SiN/Au, respectively, as shown in Fig. 1.

Silicon
SiN – 0.8 µm
ZnO – 0.66 µm
Au electrodes – 0.08 µm
Au layer – 0.1µm

Fig. 1: Cross section of the FBAR sensor.

Antibody Immobilization

To immobilize antibody on the gold layer, protein A bound to antibody is adsorbed to the gold, so a polarized bond between the gold layer and the antibody is formed [3]. Protein A (isolated from microbial pathogen *Staphylococcus aureus*) is designed to recognize and bind with high affinity to the Fc portion of an IgG subclass antibody, leaving its antigen binding sites free. Protein A ensures that the antibody is properly oriented, facing sources of antigens.

The antibody immobilization process [4] consists of two parts: (1) polarizing the gold surface and (2) coating of the antibody. Surface polarization was done by immersing the chip in the following sequence: 1.2 M HCl for 5 minutes, 1.2 M NaOH for 5 minutes and 1.2 M HCl for 2 minutes. Each immersion was followed by DI water washes. The chip was then washed in TAE (Tris-Acetate-ethylenediaminetetraacetic acid) buffer, and allowed to air dry. However, for our devices

MEMS 2008, Tucson, AZ, USA, January 13-17, 2008

with etch cavities, due to surface tension, the buffer solution tends to concentrate at the corners of the chamber on the backside. Thus, the air-drying process was accompanied with spinning the sensor chip at 500 RPM (revolutions per minute) to achieve uniform drying.

The protein A-antibody solution was prepared by dissolving protein A (0.2 mg) in 100 µl of TAE buffer, followed by mixing with 10 µl of antibody solution (0.0218 mg/ml), and placed at 4°C for 2 hours. The protein A-antibody solution was then applied to the chip, and allowed to air dry accompanied with spinning as before. Thus, we obtained an antibody layer immobilized on a sensor surface as shown in Fig. 2.
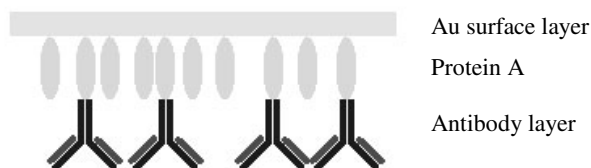

Fig. 2: Antibody immobilization on the backside of FBAR sensor.

3. TESTING SETUP

Fig. 3: Testing platform for FBAR sensor with heating element.

The testing platform shown in Fig. 3 was made with an acrylic plastic covered with a metal film for better grounding of the sensor. A patterned 1 kΩ resistor on SOI (silicon-on-insulator) wafer was used as a heating source to enhance the vaporization of explosive. Two separate compartments that were connected via a hole were used to minimize heat disturbance from the heating element. A photo of the testing setup is shown in Fig. 4.
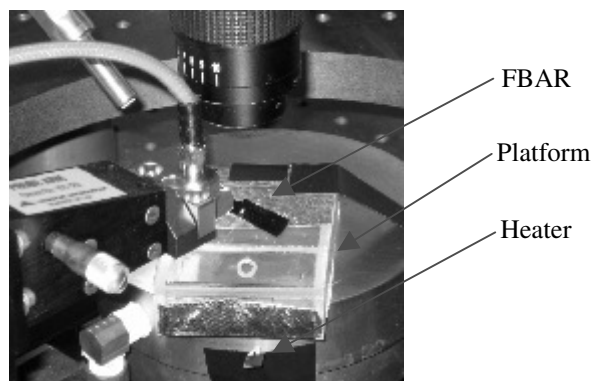

Fig. 4: Photo of the testing setup.

The FBAR's impedance characteristics were measured with a HP8753D network analyzer, and processed with LabVIEW on a computer. We have developed a LabVIEW program to track the resonant frequency where the Q is highest. A typical FBAR sensor coated with the antibody was measured to have a Q of 500 - 600 at 1.3 – 1.7 GHz as shown in Fig. 5, which gives a noise floor of 1 ppm.
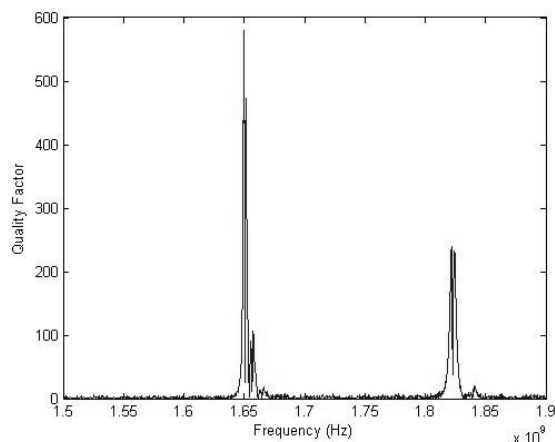

Fig. 5: Measured Q of the FBAR sensor for RDX vapor.

Since temperature fluctuation affects FBAR's resonant frequency, we took the following steps in sequence: first measure FBAR's impedance with the network analyzer and choose the frequency range to monitor, calibrate the network analyzer, reconnect the network analyzer to the FBAR, turn on the heating element, start the LabVIEW tracking program, wait until the frequency stabilizes, place explosive powder on the heating element without touching any other parts of the test setup, and then read out the resonant frequency shift.

4. RESULTS
Selection of Explosives

Among the various common explosives shown in Table 1 [5], we chose TNT and RDX for our testings due to the availability of monoclonal antibodies specifically made for them from Strategic Biosolutions.

Table 1: Vapor pressure and detection threshold of the most common explosives.

Explosives	Vapor pressure (torr) at 25°C	Detection threshold (mole concentration)
2,4,6-Trinitrotoluene (TNT)	5.8×10^{-6}	8 ppb
1,3,5-Trinitro-1,3,5-triazacyclohexane (RDX)	4.6×10^{-9}	6 ppt
Nitroglycerin (NG)	0.0025	3 ppm
Pentaerythritol tetranitrate (PETN)	5.6×10^{-8}	0.07 ppb

TNT Testing

The testing for TNT was done at room temperature. The measurement shows about 7.7 ppm frequency shift with resonant frequency of FBAR at 1.96 GHz, as shown in Fig. 6.

209

Since the TNT vapor concentration at room temperature is known to be 8 ppb, the sensor has a frequency shift of 0.96 ppm per ppb of TNT. The performance of the FBAR used in this testing was actually one with relatively low Q due to fabrication issues. With an FBAR having higher Q, we can expect a better noise floor and a smaller minimum detectable mass.
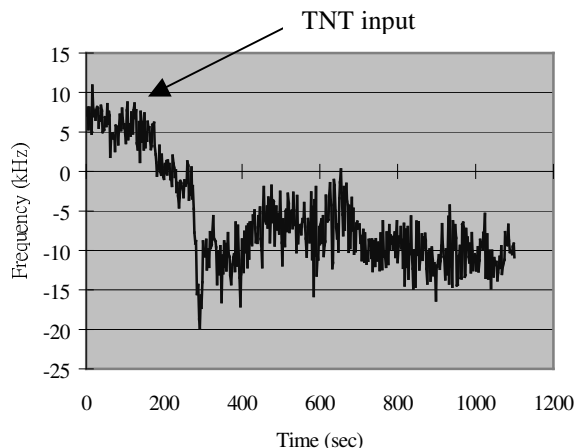

Fig. 6: TNT sensing result with frequency drift removed and zoomed in.

RDX Testing

The testing for RDX was done with RDX vapor heated to 50°C due to its lower vapor pressure compared to TNT. With the RDX heated to 50°C, we obtained 2.8 ppm frequency shift with an FBAR operating at 1.65 GHz, as shown in Fig. 7. Since the RDX vapor concentration is known to be about 270 ppt at 50°C, the measured data would correspond to a frequency shift of 10.4 ppm per ppb of RDX. This sensitivity is higher than that for TNT, possibly due to a better fabrication process for the RDX FBAR sensor than for the TNT FBAR sensor, though those had been processed very much alike.
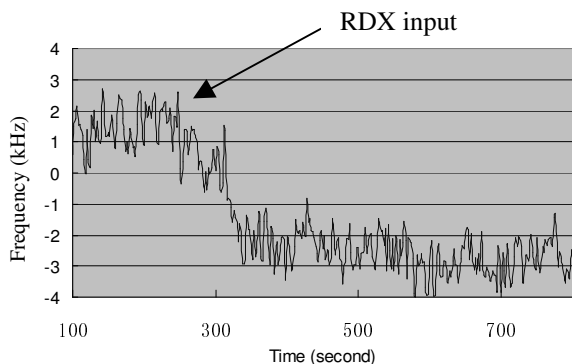

Fig. 7: RDX sensing result with frequency drift removed and zoomed in.

The vapor concentration of RDX at room temperature is given to be 6 ppt, which will produce a 62 ppb frequency shift on this FBAR sensor, according to the measured

sensitivity above. With the current noise floor, such frequency shift will be buried under the noise. However, with an FBAR having higher Q and lower TCF (temperature coefficient of frequency), the noise floor can be made to be less than 50 ppb, and the FBAR sensor will be able to detect RDX vapor at room temperature without any pre-concentrator.

Dot Blots for Verification of Antibody Quality

In order to verify the efficiency of the antigen-antibody binding, we have used dot blotting (Fig. 8) as follows. First, we laid down antigen spots (i.e., explosive compound dissolved in recommended solvent at various concentrations) on a membrane. Blocking reagent was then applied to block non-specific binding. Primary antibody (i.e., the antibody against a specific explosive that we purchased from Strategic Biosolutions) was then applied for binding to the antigen. Then, after rinsing off non-specifically adsorbed primary antibody, we applied a secondary antibody with HRP (Horseradish Peroxidase) conjugated for binding to the primary antibody. Finally, a chemiluminescent substrate was applied so that the HRP would cleave the substrate, producing light, which was exposed to X-ray film. The above steps were carried out with washing steps to eliminate non-specific binding of the antibodies to the membrane.
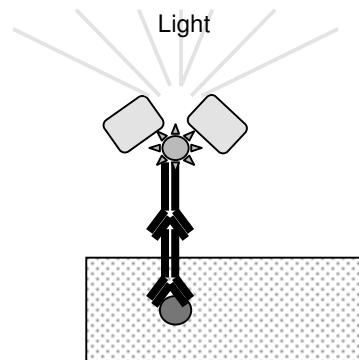

Fig. 8: Dot blot mechanism: An antigen (circular dot) is spotted and dried onto a membrane (spotted rectangle). Then a 1° antibody (lower inverted Y) specific to the antigen is bound and rinsed for specificity. Then a 2° antibody (upper inverted Y) specific to the Fc (stems of the Y structures) region of the 1° antibody and conjugated with HRP (star) is bound and rinsed for specificity. Chemiluminescent substrate (small rectangles) is then exposed to the blot. Wherever there is HRP, the substrate will be cleaved and emit light (spreading lines). Antigen = TNT, membrane = polyvinyl membrane, insoluble in acetone, 1° antibody = mouse IgG anti-TNT, 2° antibody =HRP (horseradish peroxidase) conjugated goat IgG anti-mouse, chemiluminescent substrate = Westfemto Supersignal (Pierce).

We confirmed that all of the antibodies (barring the antibodies in the presence of the explosive) did indeed function (albeit under aqueous conditions), since we were able to observe binding between the primary and secondary antibodies in the absence of the antigen as shown in Fig. 9, where anti-TNT and anti-FITC are the primary antibodies made for TNT and FITC (Fluorescein Isothiocyanate, a derivative of fluorescein), respectively. Those antibodies were indeed

observed to bind to the secondary antibodies conjugated with HRP in the dot blottings.
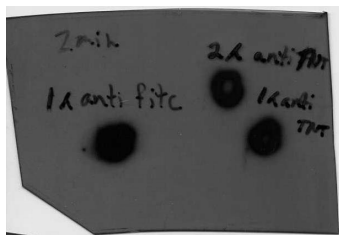

Fig. 9: Binding test between primary antibody and HRP conjugated secondary antibody.

However, we found that the TNT (dissolved in acetone) degraded the HRP-conjugated secondary antibody, causing it not to emit light isotropically as shown in Fig. 10. The experiment was done by laying down TNT solutions on the membrane, followed by drops of diluted HRP-conjugated secondary antibody. If the TNT solution were to pose no effect on the HRP-conjugated secondary antibody, we should be able to see spots as if there were no TNT solution. However, we observed that anti-spots appeared where the TNT solution was laid down on the membrane. This prevented us from verifying the antibody quality through standard dot blottings. If the TNT were dissolved in a different solvent than acetone, the damaging effect on the HRP-conjugated secondary antibody could have been avoided. We plan to explore other solvents for TNT for the standard dot blottings.
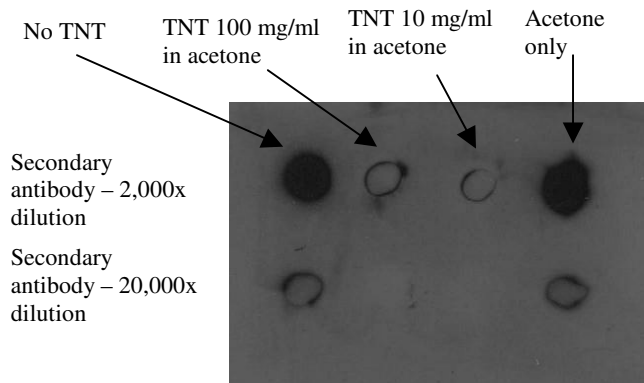

Fig. 10: Testing on TNT's effect on HRP conjugated secondary antibody.

5. CONCLUSION

We have demonstrated in this paper some preliminary results on selective detection of TNT and RDX vapor traces with FBAR-based sensors without any pre-concentrator. FBAR, with its high resonant frequency and high Q as well as small size, has excellent sensitivity and noise floor as well as great potential for extreme miniaturization. We exploit this and the concept of antigen-antibody binding to create sensors for selective detection of traces of explosives without any pre-concentrator. With the availability of antibodies made for specific explosives, it is possible to achieve sensing of those explosives with high specificity.

6. ACKNOWLEDGEMENTS

REFERENCES

[1] D. Ballantine, R. White, S. Martin, A. Ricco, E. Zellers, G. Frye, H. Wohltjen, "Acoustic Wave Sensors: Theory, Design, and Physico-Chemical Applications", Academic Press, New York, pp. 311-312, 1997.

[2] J. Bowen, L. Noe, B. Sullivan, K. Morris, V. Martin, G. Donnelly, "Gas-Phase Detection of Trinitrotoluene Utilizing a Solid-Phase Antibody Immobilized on a Gold Film by Means of Surface Plasmon Resonance Spectroscopy", *Applied Spectroscopy*, vol. 57, no. 8, pp. 906-914, 2003.

[3] L. Ghitescu, M. Bendayan, "Immunolabeling Efficiency of Protein A-Gold Complexes", *J. Histochemistry and Cytochemistry*, vol. 38, no. 11, pp. 1523-1530, 1990.

[4] S. Lee, D. Stubbs, W. Hunt, P. Edmonson, "Vapor Phase Detection of Plastic Explosives Using a SAW Resonator Immunosensor Array", *IEEE Sensors Conference*, Oct. 30 – Nov. 3, 2005, pp. 468-471.

[5] J. Morgan, W. Bryden, J. Miragliotta, L. Aamodt, "Improved Detection of Explosive Residues by Laser Thermal Desorption", *Johns Hopkins APL Technical Digest*, vol. 20, no. 3, pp. 389-395, 1999.

211