USING A CMOS-BIOMEMS CANTILEVER SENSOR FOR ORCHID VIRUS DETECTION

Lu-Hsun Cheng[1], Ya-Chun Chang[2], Wen-Chi Hu[2], Hsin-Hao Liao[3], Hann-Huei Tsai[3],Ying-Zong Juang[3] and Yen-Wen Lu[1]

[1]Dept. of Bio-Industrial Mechatronics Engineering, [2]Dept. of Plant Pathology & Microbiology, National Taiwan University, Taipei Taiwan, ROC, [3]National Chip Implementation Center (CIC), Hsinchu, Taiwan, R.O.C

ABSTRACT

We present a preliminary study on utilizing cantilever biosensors to detect *Odontoglossum ringspot tobamovirus* (ORSV) - one of the most prevalent viruses affecting the health of orchids. The cantilever surfaces of the biosensor are immobilized by ORSV-antibody (Ab) as the recognition probe for ORSV detection. Implemented by using commercially available CMOS technology and post-processed for bio-MEMS capability, the biosensors are embedded with piezoresistors, which can realize surface stress changes due to cantilever deflection coming from the specific bioaffinity between the antigen (Ag) and the antibody. The result shows -0.7% and +0.6% resistivity change when ORSV-Ab and ORSV are respectively presented.

KEYWORDS: Cantilever, Odontoglossum ringspot tobamovirus, Piezoresistor, CMOS BioMEMS

INTRODUCTION

Cantilever biosensors have been used as a sensitive and label-free diagnostic platform for malignant disease diagnosis by detecting DNA mismatch or protein biomarkers [1-3]. Figure 1 depicted the schematics of our device that contained an array of cantilevers with imbedded piezoresistors. The detection is normally achieved by modifying the surface of the cantilever with recognition molecules. The specific interaction between the recognition molecules and the target biomarkers generates surface stress and makes the deflection of the cantilever. The deflection, which is normally several nanometers, can be measured using either optical or piezoresistive readouts. In this present study, ORSV antibody (Ab) was coated onto a cantilever biosensor specifically for the detection of ORSV in plant crude saps.

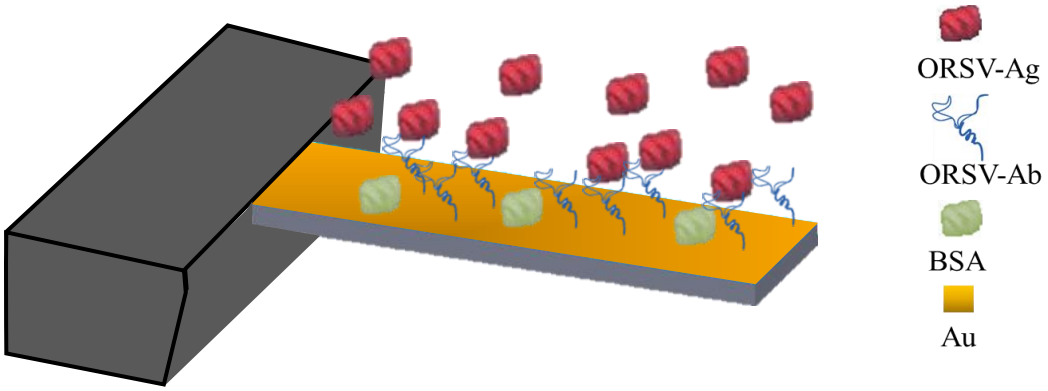

Figure 1: Schematic view of the cantilever sensors

ORSV is one of the most prevalent and economically important orchid virus, which have attained a worldwide distribution, infecting numerous commercially important orchid genera. Current ORSV detection methods include enzyme-linked immunosorbent assay (ELISA), reverse transcription-polymerase chain reaction (RT-PCR) [4], liquid chromatography (LC)- and matrix-assisted laser desorption-ionization (MALDI)-mass spectrometry [5], molecular beacons [6], quartz crystal microbalance (QCM) immunosensors [7] and immune colloidal gold [8]. While these techniques possess the advantage of highly sensitivity or qualitative determination, they are mostly performed in the laboratory environment and lack portable capability. Herein, a biosensor, implemented by commercially-available CMOS technology, is presented as a low-cost, quantitative, label-free and portable means for plant health inspection and quarantine applications.

CANTILEVER SENSOR DESIGN

The cantilever biosensor was fabricated by using TSMC 0.35μm 2P4M CMOS technology and post-processed with a gold layer for bimolecular immobilization [9]. As shown in Figure 2, the cantilever structures were composed of two metal layers (M1, M2) and two oxide (IMD, ILD) while the piezoresistors were made of n-type doped polysilicon (Poly2). The cantilever was 305μm long, 30μm wide, and 3.865μm thick with a resistance of 11.488 kΩ. The cantilever was coated with a 50-nm titanium adhesion layer and a 300nm gold layer, for post-processing. This procedure allowed the covalent bindings in the following immobilization steps. The passivation layer of the oxide and nitride, which defined the sensing area, was etched

away. Anisotropic etching was then conducted, followed by isotropic etching, releasing the cantilever structures. The overview of the cantilever array was depicted in Figure 3.

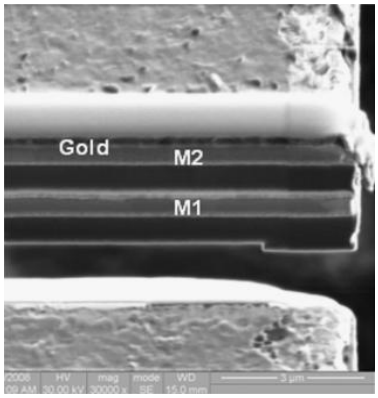

Figure 2: Cross section of CMOS BioMEMS process [9]

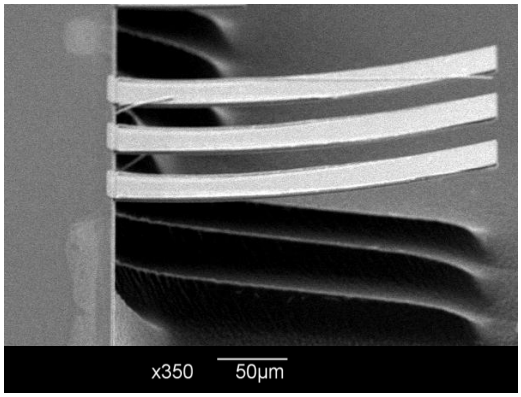

Figure 3: SEM images of the microcantilever

The sensor was then packaged onto a printed circuit broad (PCB) for testing. Polydimethylsiloxane (PDMS) as applied to keep the regions being chemically inert, and to prevent wire-bond striping and circuit shorting. The cantilever region however was PDMS-free, where biofluid samples, or health/infected orchid leaf crude saps, can be placed onto. Figure 4 illustrated the package scheme.

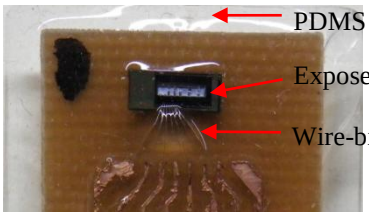

Figure 4: Biosensor packaging scheme

ANTIBODY IMMOBILIZATION AND SPECIMEN TESTING

The experimental protocol basically followed the flow chart in Figure 5. The ORSV-Ab was first immobilized onto the gold surfaces of the cantilevers. The cantilever sensors were thoroughly soaked in cross-linker, 0.2% 3,3'-dithiopropionic acid (DTP) in ethanol, for two hours at room temperature. The sensors were immersed in a mixed solution of 75 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 11.5mg/ml N-hydroxysuccinimide (NHS) for 30 minutes to form a surface self-assembly monolayer (SAM) on the gold layer. Deionized (DI) water and ethanol were then followed. The biomarker of 0.3 μg/μl ORSV-Ab in sodium phosphate buffer (PBS, 0.1M, PH=7) was then applied on the sensors. After incubation for one hour at room temperature, the cantilevers were rinsed with PBS to remove redundant antibody solution, and then immersed in ECI buffer to create a blocking layer for preventing nonspecific bindings. The cantilevers were rinsed times with PBS and followed by DI water , and then air-dried. The impedance of each cantilever was measured.

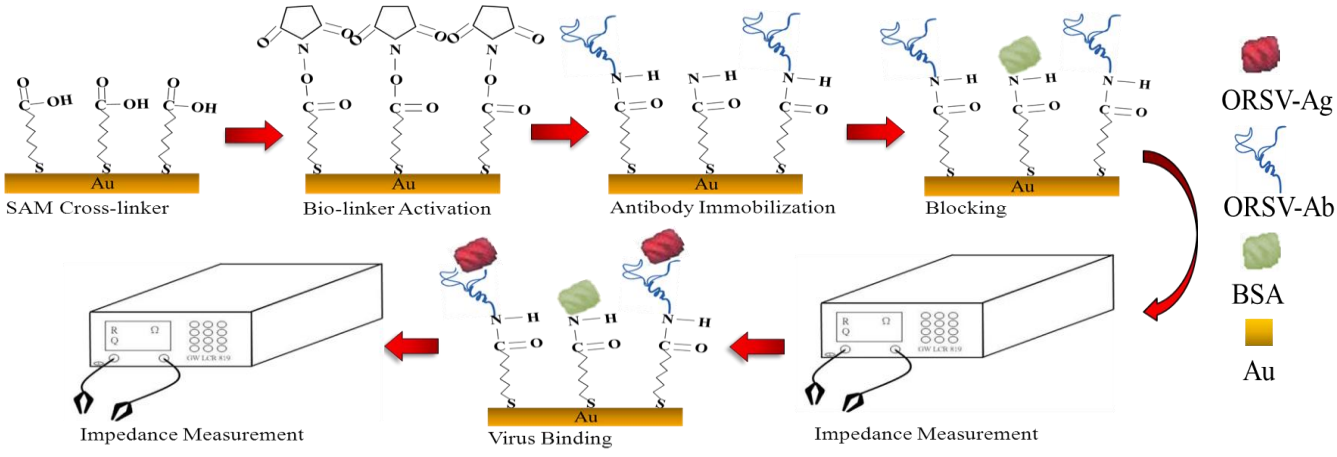

Figure 5: Flow chart of experimental protocol

Biofluid sample of 20mL in 15ng/μl ORSV in orchid leaf crude saps was applied on the sensing regions of the cantilevers and incubated for one hour at room temperature. The sensors were rinsed in PBS to remove redundant protein molecules and followed by DI water. The device was subsequently air-dried and the impedance was recorded to realize the average values of impendence change due to specific bindings.

RESULTS AND DISCUSSION

Figure 6 showed the measurement results of the sensors with biofluid samples of ORSV-Ab, ORSV-Ag and control groups, which only contain PBS. When the ORSV-Ab in PBS was immobilized on the cantilever sensors, the average values of the impedance were found to decrease from 11.488kΩ to 11.413kΩ, corresponding to a -0.7% change. Later, ORSV-Ag in orchid leaf crude saps was applied onto the sensing region; the average value of impedance increased from 11.413 kΩ to 11.481 kΩ which yield +0.6% resistivity change. The difference between experiment and control groups is about 0.5%; these results can be distinguished by using Wheatstone bridge and an instrumental amplifier.

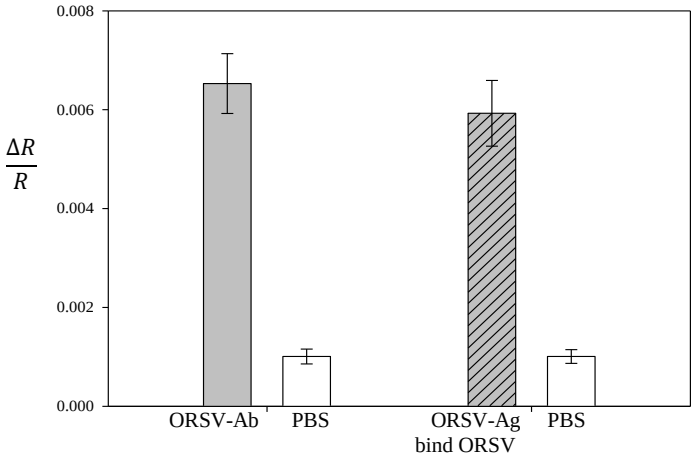

Figure 6: Resistance change of ORSV-Ab immobilization and ORSV binding comparing to PBS(control). Each value was the average values of at least three different measurements.

CONCLUSION

The cantilever biosensors, fabricated by low-cost semiconductor microfabrication process, show its potential as an ideal platform for high-throughput, label-free orchid virus (ORSV) detection. The sensors, whose resistance can change while ORSV presented, are intended for on-site orchid pathogen detection. They hold the great promise for rapid, orchid plant health inspection and can be a point-of-care (POC) device, which is advantageous compared to most of today's orchid-virus detection schemes (e.g. ELISA or RT-PCR).

ACKNOWLEDGEMENTS

The authors also thank for Mr. F.L. Lai and Dr. L.-S. Huang in Institute of Applied Mechanics (IAM) at NTU for their valuable input on surface immobilization. This work has been supported by National Science Council (NSC98-2218-E-002-023-MY3).

REFERENCES

[1] J. Fritz, M. K. Baller, H. P. Lang *et al.*, "Translating biomolecular recognition into nanomechanics," *Science,* vol. 288, no. 5464, pp. 316-318, April 14, 2000, 2000.

[2] G. Wu, R. H. Datar, K. M. Hansen *et al.*, "Bioassay of prostate-specific antigen (PSA) using cantilevers," *Nat Biotech,* vol. 19, no. 9, pp. 856-860, 2001.

[3] K. W. Wee, G. Y. Kang, J. Park *et al.*, "Novel electrical detection of label-free disease marker proteins using piezoresistive self-sensing micro-cantilevers," *Biosensors and Bioelectronics,* vol. 20, no. 10, pp. 1932-1938, 2005.

[4] A. J.-C. Eun, M.-L. Seoh, and S.-M. Wong, "Simultaneous quantitation of two orchid viruses by the TaqMan® real-time RT-PCR," *Journal of Virological Methods,* vol. 87, no. 1-2, pp. 151-160, 2000.

[5] S. W.-L. Tan, S.-M. Wong, and R. M. Kini, "Rapid simultaneous detection of two orchid viruses using LC- and/or MALDI-mass spectrometry," *Journal of Virological Methods,* vol. 85, no. 1-2, pp. 93-99, 2000.

[6] A. J.-C. Eun, and S.-M. Wong, "Molecular Beacons: A New Approach to Plant Virus Detection," *Phytopathology,* vol. 90, no. 3, pp. 269-275, 2000.

[7] A. J.-C. Eun, L. Huang, F.-T. Chew *et al.*, "Detection of two orchid viruses using quartz crystal microbalance (QCM) immunosensors," *Journal of Virological Methods,* vol. 99, no. 1-2, pp. 71-79, 2002.

[8] M. Moeremans, G. Daneels, A. Van Dijck *et al.*, "Sensitive visualization of antigen-antibody reactions in dot and blot immune overlay assays with immunogold and immunogold/silver staining," *Journal of Immunological Methods,* vol. 74, no. 2, pp. 353-360, 1984.

[9] H.-H. Tsai, C.-F. Lin, Y.-Z. Juang *et al.*, "Multiple type biosensors fabricated using the CMOS BioMEMS platform," *Sensors and Actuators B: Chemical,* vol. 144, no. 2, pp. 407-412, 2010.