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Sensitive and Label-free Detection of Cartilage Oligomeric Matrix Protein on Quartz Crystal Microbalance

Chi-Yen Shen, Chun-Lin Wu, Yu-Min Lin, Rey-Chue Hwang*

***Abstract*—Osteoarthritis (OA), the most common joint disease, is due to articular cartilage degradation, leading to joint destruction. The most common methods for diagnosing OA are done using radiographs and enzyme-linked immunosorbent assay (ELISA) method which are specific but require specialized laboratory facilities and highly trained personal. To avoid invasive methods for diagnosis, the current study describes a novel non-invasive detection method for osteoarthritis urine biomarkers. A highly selective and specific monoclonal antibodies, cartilage oligomeric matrix protein (COMP), were used to immobilize on a quartz crystal microbalance (QCM) sensor. The developed QCM sensor for COMP detection showed good sensitivity, linearity, specificity and reliability.**

***Index Terms*—osteoarthritis, quartz crystal microbalance, cartilage oligomeric matrix protein, urine.**

I. Introduction

OA is a common age-related disease that involves the slow and progressive degeneration of articular cartilage in many joints. The most widely method of diagnosing OA is measurement of the joint space width using radiographs. Plain radiograph is the most standard method used to measure the narrowing of space between joints and assess OA progression. However, radiographic endpoints lack sensitivity and only could detect significant joint damage. Furthermore, physical examination and radiographic images are unable to provide information on prognosis or progression of OA. Function exertion of specific proteins are key factors in disease progression, biological markers may be potentially more sensitive method of providing sufficient information to reveal the dynamic changes of the cartilage. Serum levels of COMP can provide important information on metabolic changes occurring in the cartilage matrix in joint diseases that correlates with cartilage degradation [1-3]. Therefore, COMP in serum detected and quantified by ELISA method has been used for cartilage degradation detection in OA [4]. However the assay always requires much time, high cost of laboratory equipment, complicated procedures and also needs special technicians.

The affinity interaction of the antibody with the specific antigen can be detected by biosensors. Considerable efforts have been directed towards the development of simple biosensors for the detection of viruses [5-8]. Piezoelectric QCM sensors are suitable for detection purposes as they are known for high precision, stable oscillators and sensitivity to sub-nanogram changes in mass [9]. Terminal functional group (i.e., COOH, OH and NH_2) of the self-assembled monolayer (SAM) on QCM sensors are used to conjugate to biological compounds, such as antibodies or probes that are capable of binding antigens or targets. Due to special structural properties and biological compatibility, sensors coated with gold enhance the sensitivity of QCM sensors [10]. The affinity interaction of the antibody with the specific antigen affects the operation frequency of the QCM. The relationship between frequency shift and mass change on the QCM surface can be described by the Sauerbrey equation $\Delta m = -C \bullet \Delta f$. The QCM consequently detect mass changes due to molecular interactions on the QCM surface. Therefore, the QCM device is convenient to use and rapidly detect real-time responses of antigen–antibody interactions on the surface of device.

Most biochemical diagnosis of cartilage degradation uses synovial fluid from invasive operation at diseased sites or serum. A non-invasive method for monitoring the cartilage degradation is attractive due to its simplicity. Accordingly, a non-invasive urination QCM sensor was developed in this work. Though many serum biomarkers have been verified to impact on OA, urine biomarkers are not certainly identified in the progressive destruction of articular cartilage. In our biomarker discovery, MS/MS identification revealed the expression level of COMP was significantly elevated in urine samples from patients with severe OA. These results suggest the possibility of COMP as a combinatorial biomarker in OA diagnosis. In this study, a simple and disposable QCM sensor was developed to quantify COMP concentration of urine for diagnosis of OA.

II. Experimental Section

A. Materials

COMP Human, Mouse Monoclonal Antibody, Clone:16F12 was purchased from BioVendor (North Carolina, USA) and Recombinant Human COMP (>90%) was purchased from R&D Systems (Minnesota, USA). N'-(3-dimethylaminopropyl)-3-ethyl carbodiimide

Chi-Yen Shen, Department of Electrical Engineering, I-Shou University, Kaohsiung City, Taiwan, R. O. C.,
Chun-Lin Wu, Department of Electrical Engineering, I-Shou University, Kaohsiung City, Taiwan, R. O. C.,
Yu-Min Lin, Department of Electrical Engineering, I-Shou University, Kaohsiung City, Taiwan, R. O. C.,
Rey-Chue Hwang, Department of Electrical Engineering, I-Shou University, Kaohsiung City, Taiwan, R. O. C.,
*Corresponding author

hydrochloride (EDC, 99%), medium for preparing phosphate buffer saline (PBS, 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). Thioctic Acid (TA, >98%) was obtained from ACROS (USA). Doubly distilled water was used throughout the experiments.

B. Immobilization of Antibody

A QCM sensor (Taitien Co., Ltd, Taiwan), coupled inside a flow injection system, was 10 MHz quartz crystal with 3.8 mm diameter gold electrode. Each of the gold electrodes was pretreated by electrochemical cleaning in 0.5 M H_2SO_4 solution using cyclic voltammetry at a scan rate of 100 mV/s for 5 cycles and then washed in de-ionized water and dried with a light stream of nitrogen gas. The pretreated gold electrode was immersed in the 2.5 mM thioctic acid (TA) alcohol solution at room temperature for 24 hr in the darkroom. Afterwards, it was rinsed thoroughly with ethanol and dried with nitrogen gas and stock at room temperature for further used.

The coupling agents, 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was used to activated the prepared TA monolayer for 3 hr at room temperature and then rinsed with ethanol and dried as aforementioned. Then 20 µl of COMP monoclonal antibody (0.01 mg ml-1) in PBS solution was placed on the electrode to conjugate at 4°C for 12 hr and then rinsed by PBS. Afterwards, the electrode was blocking by 5% bovine serum albumin (BSA) for 1.5 hr. Finally, the electrode was rinsed with PBS and then dried by nitrogen gas.

C. QCM Measurement

The prepared QCM sensor was mounted on one side of the detection vessel. PBS solution with pH 7.4 was prepared to be an assay buffer solution and was injected into the vessel to stabilize the equipment. A frequency counter collected the resonance frequency of the oscillator. After stabilization of the resonance frequency of QCM, 4 mL COMP solution was then introduced into the detection vessel. The resonance frequency was recorded during the immunoreactions until equilibrium was reached 25 min in order to avoid the response induced by non-specific adsorption.

The shifts of the resonance frequency in all experiments were calculated on the average responses of the immunoreactions with corresponding standard deviations of triplicate measurements.

III. Results and Discussion

The immobilization and detection of COMP on QCM sensor resulted in frequency shifts. Fig. 1 shows the typical responses of frequency for COMP immobilization and detection. It indicates the successful immobilization and detection of COMP by using QCM sensor. The binding capacity of the proposed QCM sensor is examined by detecting various concentrations of COMP. Fig. 2 shows the typical frequency responses monitored by the QCM sensor for COMP detecting at 26°C. A QCM sensor with COMP antibodies is simple, more rapid and more sensitive than commercialized ELISA kits systems. Fig. 3 illustrates a linear relationship between 1-10 ng/ml COMP and frequency shift. The linear regression equation of frequency shift and COMP concentration was determined to be: y = 3.9778x + 12.478. A QCM sensor with a COMP monoclonal antibody improves sensitivity for COMP detection (as low as 1 ng/mL).

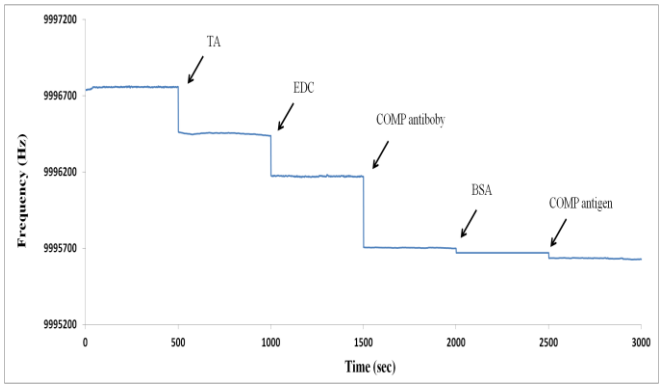


Fig. 1. The typical response of QCM sensor for COMP immobilization and detection.

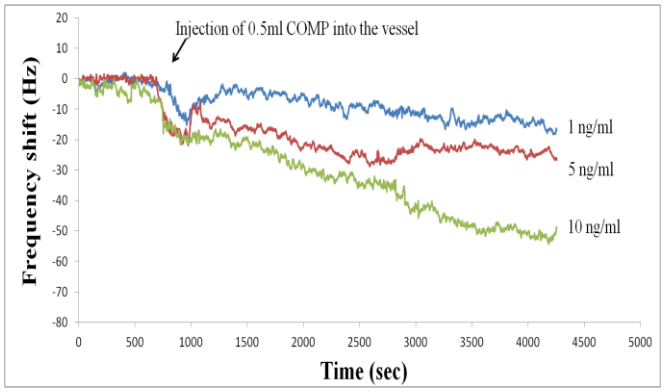


Fig. 2. Frequency responses of the developed QCM sensor for detecting COMP.

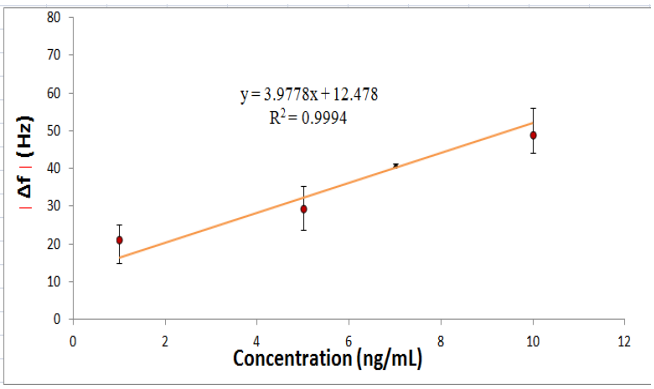


Fig. 3. Calibration curve: Relationship between COMP concentration and observed frequency shift.

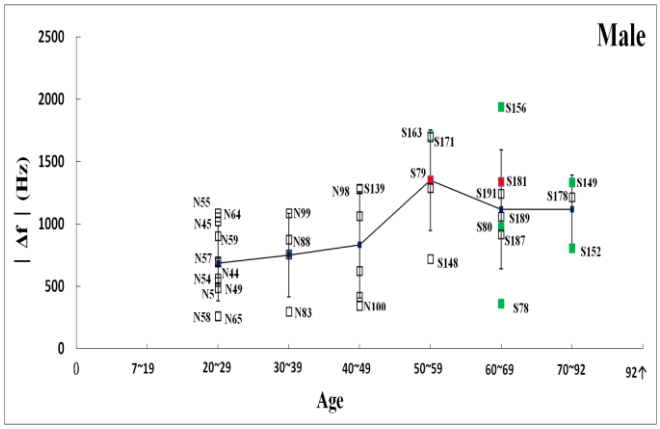
Table 1 shows that two additional concentrations of COMP, 4 ng/ml and 6 ng/ml, were examined under the same procedures and later fitted onto the calibration curve. The frequency shifts measured by QCM sensor were 22.4 Hz and 36.8 Hz at 4 ng/ml and 6 ng/ml COMP concentrations, respectively. Compare to the linear regression equation shown in Fig. 3, these results suggested the developed QCM sensor shows sensitive and reliable detection properties.

An interference frequency from 10 ng/ml BSA was detected by QCM at approximately 3.5 Hz, indicating low noise signal background. In response to 1 ng/ml COMP, the frequency response was 14.8 Hz and the noise level was 4.5 Hz. Therefore, the estimated detection limit at signal-to-noise ratio of two was 0.61 ng/ml of COMP in this work.

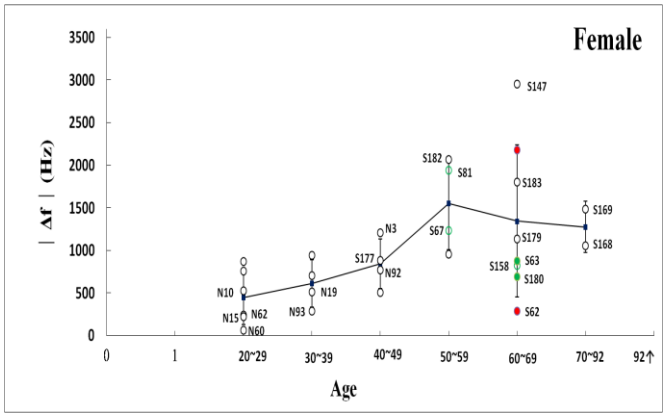
To evaluate the sensitivity of the QCM sensor coated with the COMP monoclonal antibody, urine from 54 volunteers (26 men and 26 women) was analyzed. In Fig. 4(a) and (b), COMP concentrations in urine increased with age, revealing that the same concentration distributions of the COMP in serum that were recorded on the datasheet of ELISA kit (BioVender) [11]. Further analysis of a greater number of urine specimens is required and will help to establish the distributions of COMP concentration on ages and genders. Otherwise, if the patients had treated by surgery operation (labeled S78 and S62 in Fig. 4) there was no or less cartilage to be destroyed, the COMP concentration in urine would decrease immediately.

Table 1: Reliability of the QCM sensor.

COMP (ng/ml)	Measured frequency shift (Hz)	Predicted frequency shift (Hz)
4	22.4	28.4
6	36.8	36.3



(a)



(b)

Fig. 4. Detection of the urine COMP level of 54 volunteers' urine samples by QCM sensor. Data from OA patients are shown in green and red symbols.

IV. Conclusion

The QCM sensor developed in this study showed a mass-sensitivity and sensing linearity for COMP detection. No interference was found which indicated the specific binding on the sensor. These QCM sensor will be more quick and easy to operate and will help for OA diagnosis. Early diagnosis of OA is important for cartilage preservation and medical treatment. Results from the current study support the potential application of QCM biosensors for detection of cartilage degradation in OA patients.

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References

[1] T. C. B. Pollard, S. E. Gwilym, & A. J. Carr, "The assessment of early osteoarthritis," *J. of Bone and Joint Surgery*, vol. 90, pp. 411-421, 2008.

[2] O. Bruyere, J. H. Collette, O. Ethgen, L. C. Rovati, G. Giacovelli, Y. E. Henrotin, L. Seidel, & Y. J. Reginster, "Biochemical markers of bone and cartilage remodeling in prediction of longterm progression of knee osteoarthritis," *J. Rheumatol.*, vol. 30, pp. 1043-1050. 2003.

[3] K. Misumi, V. Vilim, P. D. Clegg, C. C. Thompson, & S. D. Carter, "Measurement of cartilage oligomeric matrix protein (COMP) in normal and diseased equine synovial fluids," *Osteoarthritis Cartilage*, vol. 9, pp. 119-127, 2001.

[4] F. Zaucke, R. Dinser, P. Maurer, & M. Paulsson, "Cartilage oligomeric matrix protein (COMP) and collagen IX are sensitive markers for the differentiation state of articular primary chondrocytes," *Biochem. J.*, vol. 358, pp. 17-24, 2001.

[5] V. Vilim, Z. Voburka, R. Vytasek, L. Senolt, I. Tchetverikov, V. B. Kraus, & K. Pavelka, "Monoclonal antibodies to human cartilage oligomeric matrix protein: epitope mapping and characterization of sandwich ELISA," *Clin. Chim. Acta.*, vol. 328, pp. 59-69, 2003.

[6] P. Critchley, N. J. Dimmock, "Binding of an influenza A virus to a neomembrane measured by surface plasmon resonance. Bioorg," *Med. Chem.*, vol. 12, pp. 2773-2780, 2004.

[7] V. M. Mecea, "From quartz crystal microbalance to fundamental principles of mass measurements," *Anal. Lett.*, vol. 38, pp. 753-767, 2005.

[8] T. Z. Wu, C. C. Su, L. K. Chen, H. H. Yang, D. F. Tai, & K. C. Peng, "Piezoelectric immunochip for the detection of dengue fever in viremia phase," *Biosens. Bioelectron*, vol. 21, pp. 689-695, 2005.

[9] C. K. O'Sullivan, G. G. Guilbault, "Commercial quartz crystal microbalances-theory and applications," *Biosens Bioelectron.*, vol. 14, pp. 663-670, 1999.

[10] T. Liu, J. Tang, L. Jiang, "The enhancement effect of gold nanoparticles as a surface modifier on DNA sensor sensitivity," *Biochem Biophys Res Commun.*, vol. 313, pp. 3-7, 2004.

[11] Human cartilage oligomeric matrix protein ELISA, Datasheet, BioVendor. pp. 17.

Chi-Yen Shen, received a B.S. degree in Electrical Engineering from Cheng-Kung University in Taiwan in 1987, Ph.D. degree in Electrical Engineering from National Sun Yat-Sen University in Taiwan in 1991. In 1991, she was employed as an associate professor of Electrical Engineering at Kaoshiung Ploytechnic Institute, Taiwan. She is currently a professor of Electrical Engineering at I-Shou University, Taiwan. Dr. Shen is a Fellow of IET and a senior member of IEEE. Her research interests are in acoustic wave devices, chemical sensors and bio-sensing technology.

Chun-Lin Wu was a master student of Electrical Engineering Department, I-Shou University. His research interests include the areas of acoustic wave devices, chemical sensors.

Yu-Min Lin was a master student of Electrical Engineering Department, I-Shou University. His research interests include the areas of acoustic wave devices and bio-sensing technology.

Rey-Chue Hwang, received his Ph.D degree in Electrical Engineering from Southern Methodist University, Dallas, TX, in 1993. Currently, he is a full professor of Electrical Engineering Department, I-Shou University, Taiwan, R.O.C. Dr. Hwang has published more than 250 papers in various journals and conferences in the areas of artificial intelligent system, signal processing and fuzzy control. He is now a Fellow of IET and a senior member of IEEE. He chartered the IEEE CIS Chapter, Tainan Section and served as the co-chair and chair from year 2004 to year 2009.