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Determination of Cyclic Citrullinated Peptide Antibodies on Quartz Crystal Microbalance Based Nanosensors

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***Abstract*—**In this study, we have focused our attention on combining of molecular imprinting into nanofilms and QCM nanosensor approaches and producing QCM nanosensor for anti-CCP, chosen as model protein, using anti-CCP imprinted nanofilms. The nonimprinted nanosensor was also prepared to evaluate the selectivity of the imprinted nanosensor. Anti-CCP imprinted QCM nanosensor was tested for real time detection of anti-CCP from aqueous solution. The kinetic and affinity studies were determined by using anti-CCP solutions with different concentrations. The responses related with mass shifts (Δm) and frequency shifts (Δf) were used to evaluate adsorption properties. To show the selectivity of the anti-CCP imprinted QCM nanosensor, competitive adsorption of anti-CCP and IgM was investigated. The results indicate that anti-CCP imprinted QCM nanosensor has higher adsorption capabilities for anti-CCP than for IgM, due to selective cavities in the polymer structure.

***Keywords*—**Anti-CCP, molecular imprinting, QCM nanosensor, rheumatoid arthritis.

I. Introduction

RHEUMATOID arthritis (RA) which is the most common autoimmune disorder of the body's own immune system attacking healthy cells. RA has both articular and systemic effects. Until now romatiod factor (RF) assay is used the most commonly diagnosed RA but it is not specific. Anti-cyclic citrullinated peptide (anti-CCP) antibodies are IgG autoantibodies which recognize citrullinated peptides and offer improved specificity in early diagnosis of RA compared to RF. Anti-CPP antibodies have received special attention, since they may be helpful for RA diagnosis; they are moderately sensitive but highly specific to RA, with a specificity that is higher than that of RF. Anti-CCP antibodies have specificity for the diagnosis of RA from 91 to 98% and the sensitivity rate of 41-68% [1]-[4].

Molecularly imprinted polymers (MIP) are materials that are easy to prepare, less expensive, stable, have talent for molecular recognition and also can be manufactured in large quantities with good reproducibility. Molecular recognition-based adsorption techniques have received much attention in several fields because of their high selectivity for target molecules [5]-[7].

Quartz crystal microbalance (QCM) is an effective, simple, inexpensive approach mass changes that can be converted into an electrical signal. The applications for specific determination of chemical substances or biomolecules, crystal electrodes, cover by the thin films for bind or adsorption of molecules [8], [9]. QCM nanosensors, member of mass-sensitive chemical sensors, have been getting researchers' attention because of their properties such as high selectivity, low cost, portability, stability and simplicity [10]. The QCM allows dynamic monitoring of biochemical interactions, using an oscillating crystal with the biomolecules immobilized on its surface. The increased mass, associated with the binding reaction, results in a decrease of the oscillating frequency [11]. Recently, QCM-based nanosensors have been used in the detection of several analytes such as clinical targets, environmental contaminants, marker of genetic diseases, determination of oxidative stress, quantification of protein, detection of genetically modified organisms (GMOs) [12]-[14].

II. Experimental

A. Modification of QCM Nanosensors Surfaces with Allyl Mercaptan

As shown in Fig. 1, the modification of QCM nanosensors was carried out with allyl mercaptan (CH_2CHCH_2SH). Before the modification, QCM nanosensors surfaces were cleaned with acidic piranha solution (3:1, H_2SO_4:H_2O_2, v/v), then washed with deionized water and ethanol, respectively, and dried at vacuum incubator. Then, allyl mercaptan was dropped onto the QCM nanosensors surfaces and incubated for 12 h in a sealed container in order to introduce allyl groups onto the nanosensors surfaces. After the modification, QCM nanosensors were rinsed with ethanol to remove unbound allyl mercaptan molecules and dried at vacuum incubator.

B. Preparation of Anti-CCP/Acrylamide Precomplex

To prepare anti-CCP/acrylamide (AA) precomplex, 45 µL anti-CCP and 21 mg AA dissolved in 500 µL water was stirred in 30 min with the help of magnetic stirrer. To define optimum template molecule and monomer ratio, anti-CCP and AA mixed in different ratios and optimum ratio was determined by using UV-visible region spectrophotometry.

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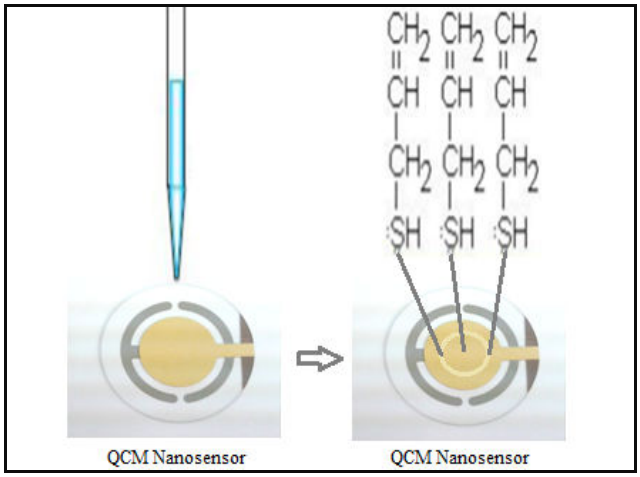

Fig. 1 Schematic representation of modification of QCM nanosensors surfaces with allyl mercaptan

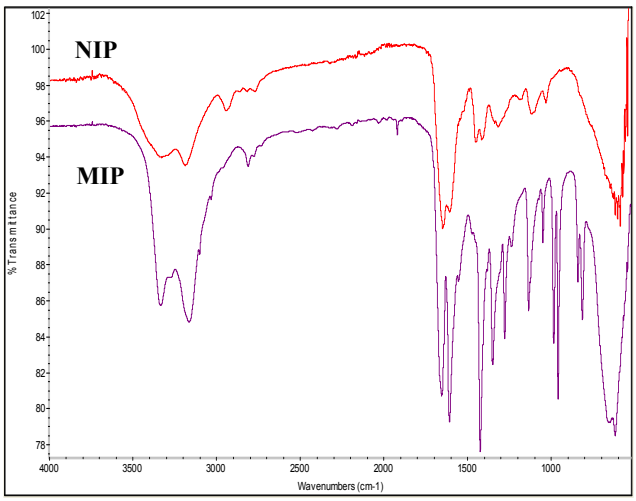

Fig. 2 FTIR-ATR spectra of the nonimprinted (NIP) anti-CCP and imprinted (MIP) QCM nanosensors

C. Preparation of Anti-CCP Imprinted QCM Nanosensor

QCM nanosensor was prepared by using precomplex and MBAAm as crosslinker. After addition 2 µL (%10) APS and 2 µL TEMED as an initiator/activator pair, allylated QCM nanosensor was coated uniformly by spin coating. The polymerization was carried out under UV light by photopolymerization method for 30 min. At the end of polymerization, the unreacted monomers and impurities were removed by methyl alcohol and dried with N_2 gas at room temperature. The nonimprinted QCM nanosensor was synthesized by applying the same procedure without addition of the template, anti-CCP.

III. Results

A. Characterization

Anti-CCP imprinted and nonimprinted QCM nanosensors were characterized by Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR), atomic force microscopy (AFM), contact angle measurements and ellipsometry.

As seen in FTIR-ATR spectra, the most important adsorption band at 3360 cm^{-1} represents ν(N-H) asymmetric stretching band, respectively. The FTIR-ATR bands observed at 2927 cm^{-1} and 1455 cm^{-1} were assigned to the aliphatic stretchings of ν(-CH_3) and ν(C=O), respectively. Other bands were the asymmetric and symmetric bands ν(COOH) at 1565 cm^{-1} and at 1416 cm^{-1}. The ν(C-N) vibration band was observed at 1253 cm^{-1}. The disappearance of the band of monomer at 1633 cm^{-1} showed that the polymerization has successfully performed.

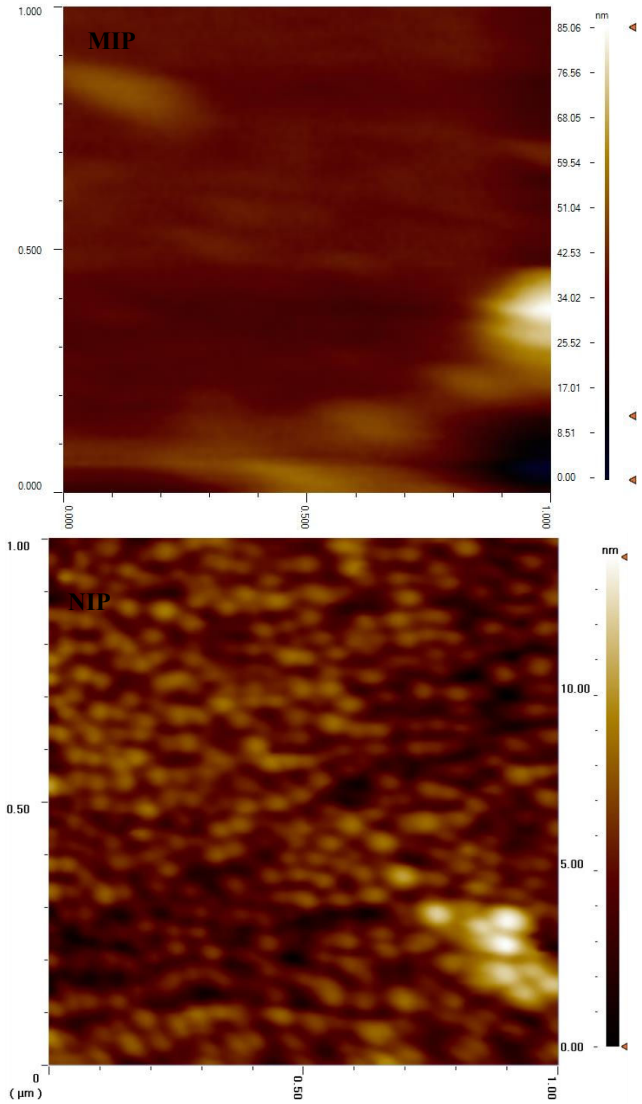

Fig. 3 AFM images of the anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM nanosensors

The anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM nanosensors were characterized by AFM (Fig. 3). Surface deepnesses determined by AFM measurements of the anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM were

26.38 nm and 18.55 nm with thicknesses 3.34 nm and 0.95 nm. Also, surface depths obtained from ellipsometry of the anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM were 94.4 ± 0.7 nm and 90.0 ± 0.9 nm in Fig. 5. As a conclusion, it can be deduced that homogeneous and monolayer attachment of the nanofilm has been accomplished.

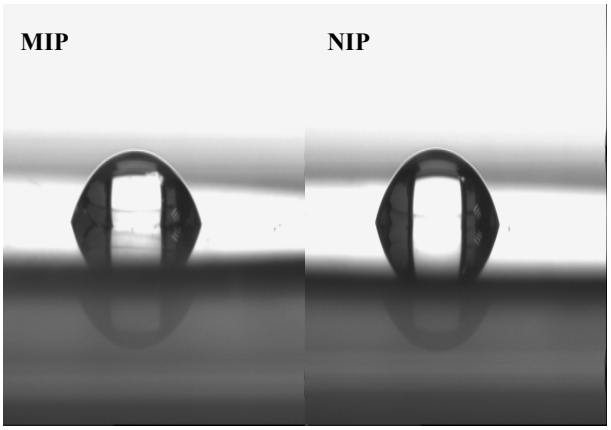

Fig. 4 Contact angle images of the anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM nanosensors

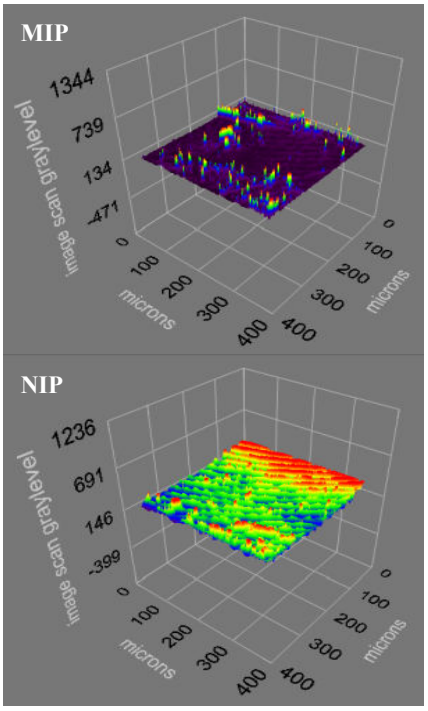

Fig. 5 Ellipsometry images of the anti-CCP imprinted (MIP) and nonimprinted (NIP) QCM nanosensors

TABLE I
CONTACT ANGLE MEASUREMENTS OF THE ANTI-CCP IMPRINTED (MIP) AND NONIMPRINTED (NIP) QCM NANOSENSORS

	MIP	NIP
Contact Angle, °	74.2	79.8

As seen in Table I, the contact angle of the nonimprinted (NIP) QCM nanosensor decreased from 79.8° to 74.2° when the hydrophilic template monomer, anti-CCP, added to polymerization mixture to prepare the anti-CCP imprinted (MIP) QCM nanosensor. Reduction of the contact angle showed that the increased hydrophilic property of the surface of nanosensor (Fig. 4).

B. Selectivity Experiment

Selectivity experiment is one of the most crucial parameter for determining the selectivity of the imprinting process. Selectivity of anti-CCP imprinted QCM nanosensors was investigated by using immunoglobulin M (IgM) in pH 7.0 phosphate buffer. Table II demonstrated that anti-CCP selectivity was 3.8 times higher IgM for anti-CCP imprinting (MIP) QCM nanosensor. Results showed that the cavities formed in the anti-CCP imprinting (MIP) QCM nanosensor specially recognized anti-CCP, indicating that cavities matched the size of IgM.

TABLE II
SELECTIVITY COEFFICIENT OF THE ANTI-CCP IMPRINTING (MIP) QCM NANOSENSOR

	MIP	
	Δm	k
Anti-CCP	0.412	-
IgM	0.108	3.815

C. Kinetic Analysis

Kinetic analysis was evaluated by using different concentration anti-CCP solutions. Δf and Δm sensograms of the interaction between the anti-CCP imprinted (MIP) QCM nanosensor and anti-CCP solution were shown in Figs. 6 and 7. While 10 mM pH 7.0 phosphate buffer was used for adsorption studies, desorption studies was carried out by using 1% Tween 20 and 10% acetic acid (HAc) containing solution.

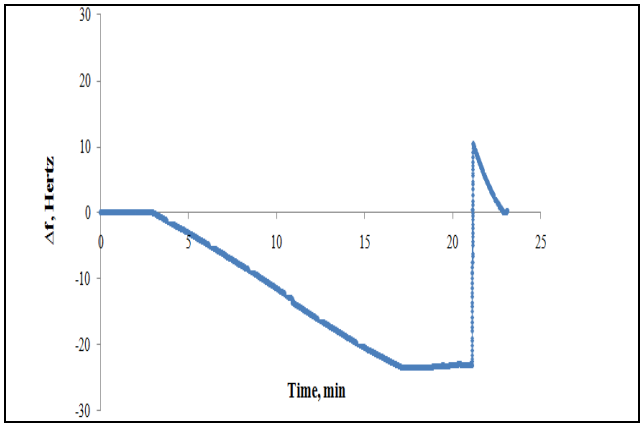

Fig. 6 Δf sensogram of the interaction between anti-CCP imprinted (MIP) QCM nanosensor and anti-CCP solution

D. Future Planning

Kinetic analysis will be evaluated by studying concentration effect on nanosensor adsorption capacity. Association (K_a) and diccociation constants (K_d) will be determined to estimate

affinity strength. To determine the adsorption model of interaction between anti-CCP solution and anti-CCP QCM nanosensor, four different adsorption models such as Scatchard, Langmuir; Freundlich and Langmuir-Freundlich (LF) will be performed. Finally detection limit (LOD) calculation will be performed.

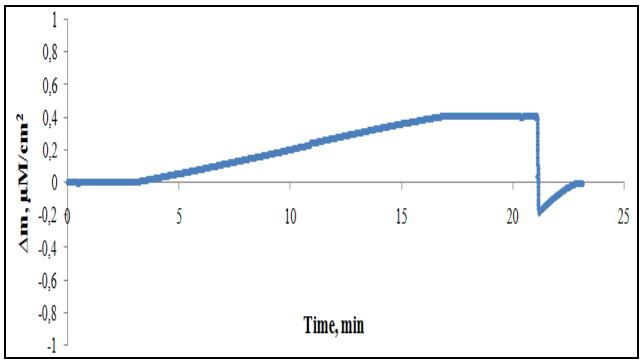

Fig. 7 Δm sensogram of the interaction between anti-CCP imprinted (MIP) QCM nanosensor and anti-CCP solution

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