Biosensors and Bioelectronics 99 (2018) 325–331

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

An ultrasensitive micropillar-based quartz crystal microbalance device for real-time measurement of protein immobilization and protein-protein interaction

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ARTICLE INFO

Keywords:
QCM
Micropillar
PMMA
Physical adsorption
Mass sensitivity improvement
Affinity

ABSTRACT

A new sensing device was developed to achieve ultrahigh sensitivity, by coupling polymer micropillars with a quartz crystal microbalance (QCM) substrate to form a two-degree- of-freedom resonance system (QCM-P). The sensitivity of these QCM-P devices was evaluated by measuring mass changes for both deposited gold film and adsorption of bovine serum albumin (BSA), respectively, on poly(methyl methacrylate) (PMMA) micropillar surfaces, as well as assessing ligand-analyte binding interactions between anti-human immunoglobulin G (anti-hIgG) and human immunoglobulin G (hIgG). The anti-hIgG and hIgG binding results show QCM-P achieved an eightfold improvement in sensitivity relative to conventional QCM sensors. In addition, the binding affinity obtained from the QCM-P device for anti-hIgG and hIgG proteins was found in good agreement with that measured by surface plasmon resonance (SPR) for the same binding reaction.

1. Introduction

Biomolecular interaction analysis (BIA), in real time, is a powerful technique for applications such as drug discovery (Cooper, 2002) and drug development (Zimmermann et al., 2002), proteomics (Issaq et al., 2002), immunogenicity and food analysis (Shankaran et al., 2007), etc. As a result, over the past two decades, the development of sensing techniques, which achieve fast and accurate measurement of the protein bindings at solid-liquid interfaces, has rapidly increased in both scientific and practical importance (Gray, 2004; Nel et al., 2009). Different techniques including optical (Hall, 2001; Lee et al., 2001), radioactive (Wolterbeek and van der Meer, 1996), electrochemical (Thévenot et al., 2001; Wang et al., 1996), piezoelectric (Ebersole et al., 1990; Horáček and Skládal, 1997), magnetic (Connolly and St Pierre, 2001), micromechanical (Raiteri et al., 2001) and mass spectrometric (Polla et al., 2000) have been developed for this purpose. Among them, surface plasmon resonance (SPR) (Mrksich et al., 1995), an optical based system, has become the dominant sensing technology for a wide range of BIA applications, such as the adsorption of small molecules (Jung and Campbell, 2000a, 2000b), ligand-receptor binding (Mann et al., 1998; Pérez-Luna et al., 1999), antibody-antigen binding (Berger et al., 1998), DNA and RNA hybridization (Jordan et al., 1997; Nelson et al., 2001) and protein-DNA interactions (Brockman et al., 1999). However, SPR system costs, and requirements of well-trained technical experts and expensive materials, raise significant barriers to wider use. Quartz crystal microbalance (QCM), as a piezoelectric sensor, is an attractive technique for biological and biochemical research, due to its simplicity and low cost (Sagmeister et al., 2009; Speight and Cooper, 2012). The resonance frequency of QCM shifts to lower value in response to an increase in mass attaching to its surface, while viscosity change on that surface can be exploited for biomolecular interaction analysis (Stadler et al., 2003). In addition, QCM has much greater flexibility with respect to the range of samples that can be used, because the system operates acoustically, rather than optically, thus allowing direct measurement of, for example, biomolecular interactions (BIA) in serum.

For a conventional QCM device, the relationship between the resonant frequency shift and the mass attached to the sensing surface, Δm, is described by the Sauerbrey equation (Sauerbrey, 1959) as

$$\Delta f = S_Q \frac{\Delta m}{A} \tag{1}$$

where S_Q is the mass sensitivity constant, presented as

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http://dx.doi.org/10.1016/j.bios.2017.07.074
Received 8 May 2017; Received in revised form 27 July 2017; Accepted 30 July 2017
Available online 02 August 2017
0956-5663/

$$S_Q = \frac{2f_0^2}{\sqrt{\rho_q \mu_q}} \tag{2}$$

where f_0 is the fundamental resonant frequency of the QCM without any mass loading, while ρ_q and μ_q are the density and shear modulus of quartz crystal, respectively. Therefore, the correlated mass sensitivity constant (S_Q) is 0.226 Hz/ (ng cm^{-2}), for a 10 MHz QCM.

The major drawback of a conventional QCM device is its relatively low sensitivity, which is about one order of magnitude lower than SPR (Su et al., 2005). Considering the requirement of extremely high sensitivity (100 pg/cm^2) for most BIA applications, use of conventional QCM sensors in this field remains a challenge. Based on the expression of S_Q in Eq. (2), a higher mass sensitivity is achievable by operating the QCM sensor at a higher fundamental frequency, f_o, which is enabled by reducing the thickness of QCM quartz crystal substrate from hundreds to tens of microns. For instance, a high fundamental frequency (HFF) QCM biosensor was first demonstrated by Uttenthaler (Uttenthaler et al., 2001). However, HFF QCM resonators are not very practical, due to their fragileness (Ogi et al., 2009; Sagmeister et al., 2009) and high cost. Furthermore, there is difficulty in obtaining a stable resonance frequency signal from HFF QCM sensors because of their extreme susceptibility to hydrostatic pressure fluctuation in solution (Sota et al., 2002), which lowers HFF QCM sensitivity accordingly.

Recently, a new sensing mechanism was discovered, by coupling polymer micropillars with QCM to form a two-degrees-of-freedom resonant vibration system (QCM-P) having significant improvement in sensitivity over conventional QCM sensors (Wang et al., 2014a, 2015, 2014b). This makes QCM-P a viable technique for label-free detection of protein binding reaction (Cooper, 2003). Furthermore, as these micropillars are fabricated on QCM substrates using nanoimprint lithography (NIL), large-scale manufacture of QCM-P sensors become possible. The main objective of this work is to demonstrate a new type of low cost, ultrahigh sensitivity QCM-P biosensing device, for measurement of protein adsorption and ligand-analyte binding kinetics.

A theoretical model was initially developed to understand the ultrahigh sensitivity manifested by QCM-P devices. Thereafter, QCM-P devices with well-controlled micropillar dimensions and materials were fabricated using NIL. Next, the sensitivities of QCM-P devices with different PMMA pillar heights and the conventional PMMA film-based QCM (QCM-F) were evaluated by using (1) well-controlled thin gold film deposition, and (2) the physical adsorption of BSA onto respective sensor surfaces. These experimental results were then compared with predictions from our theoretical model, and good agreement was found. Finally, a QCM-P device with optimized pillar height was used to measure ligand-analyte binding kinetics. A direct comparison between QCM-P and conventional QCM-F was conducted through measurement of reaction affinity between anti-hIgG and hIgG in a flow cell system. For validation purposes, the binding affinity (K_D) value obtained from QCM-P device was compared with that measured by a surface plasmon resonance (SPR) device for the same ligand-analyte reaction.

2. Sensing mechanism of QCM-P devices

The micropillar and QCM substrate form a two-degree-of-freedom resonator in series, as shown in Fig. 1.

The QCM substrate is considered as an equivalent mass-spring system with mass (M_q) and a spring with force constant (k_q). A PMMA micropillar is treated as the second component (mass of M_p and spring constant of k_p). Detailed information about M_p and k_p can be found in supplementary materials. Therefore, the relationship between displacement of the QCM and micropillar can be established based on Newton's second law as:

$$\begin{bmatrix} M_q & 0 \\ 0 & M_p \end{bmatrix} \begin{pmatrix} \ddot{x}_1 \\ \ddot{x}_2 \end{pmatrix} + \begin{bmatrix} k_q + k_p & -k_p \\ -k_p & k_p \end{bmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \tag{3}$$

where x_1 and x_2 represent the displacements of the QCM and PMMA micropillar, respectively. Therefore, the resonance frequency of the QCM-P can be obtained as:

$$f = \frac{1}{2\pi} \sqrt{\frac{1}{2}\left(\frac{k_q}{M_q} + \frac{k_p}{M_q} + \frac{k_p}{M_p}\right) \pm \frac{1}{2}\left[\left(\frac{k_q}{M_q} + \frac{k_p}{M_q} + \frac{k_p}{M_p}\right)^2 - 4\frac{k_q}{M_q}\frac{k_p}{M_p}\right]^{1/2}} \tag{4}$$

After bio-molecule immobilization on PMMA micropillar surfaces, the resonance frequency of QCM-P shifts to lower value according to the attached mass, as illustrated in Fig. 1.

The resonant frequency shifts and sensitivity of QCM-P devices with varying heights of PMMA micropillar are calculated based on this simplified dynamic model. To facilitate data analysis, the resonant frequency shift of QCM-P device was normalized to the fundamental frequency of the bare QCM, as shown in Fig. 2.

Fig. 2(a) presents the theoretical predictions and experimental measurements of normalized frequency shifts for QCM-P devices of different pillar heights, operating in air and water. The frequency shift of the QCM-P device initially decreases linearly with increasing pillar height, which is consistent with Sauerbrey theory. However, different from a film-based QCM, a sudden “dip and jump” behavior appears in the so-called “ultrasensitive zone”, which is near the “critical height” (H_c), due to the coupled resonance of QCM and micropillars. When the micropillar height is less than the critical height, it acts as an inertial loading, resulting in a negative resonant frequency shift. When micropillar height approaches H_c, resonance takes place. If the coupled vibration produces a phase shift of less than 90°, QCM vibration is dampened by movement of the pillars on its surface, resulting in drop-off of the negative frequency shift. When the acoustic phase shift nears 90°, phase veering occurs, leading to a sharp positive frequency shift (normalized to the natural frequency), and is seen as a sudden upward spike in the curve. It is also worth noting, for bio-based applications, that when QCM-P -is employed in an aqueous environment, coupling between the micropillars and the surrounding liquid results in a reduction of critical height from 15 μm in air to 13 μm, due to water's hydrodynamic loading (Wang et al., 2015). We believe that discrepancies between theoretical modeling and experimental measurement in Fig. 2(a) may result from assuming that residual layer thicknesses of all the pillars are equal for all heights in theoretical modeling (Esmaeilzadeh et al., 2015).

To evaluate the mass sensitivity of QCM-P devices, a 10 Å gold layer was deposited uniformly on QCM-P devices with micropillar heights ranging from 5 to 26 μm, respectively, using an E-Beam evaporator (Solution 5720, CHA Industries). The admittance spectrum for each QCM-P device was measured by a network analyzer (HP8753C, Agilent Tech.) both before and after gold deposition, in water. The sensitivity enhancement of QCM-P when compared with QCM-F in water is shown in Fig. 2(b) based on the measurement for deposited thin gold layer and theoretical prediction. Good agreement between experimental measurements and theoretical predictions was achieved by taking into account the effects of residual layer as an additional mass. Also, up to one order of magnitude improvement in mass sensitivity is possible by QCM-P over QCM-F in the ultrasensitive zone. It should be noted that QCM-F shows identical mass sensitivity to bare QCM (0.226 Hz per ng/cm^2), as the acoustic phase shift (φ) for QCM-F is small (φ = *0.15*) (Ballantine et al., 1996). In addition, the good agreement seen between experimental measurement and theoretical prediction supports our theoretical model.