Biosensors and Bioelectronics 24 (2008) 638–643

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Micro-machined piezoelectric membrane-based immunosensor array

Ting Xu [a], Zhihong Wang [a], Jianmin Miao [a,*], Ling Yu [b], Chang Ming Li [b]

[a] Micromachines Centre, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore
[b] School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 639798, Singapore

ARTICLE INFO

Article history:
Received 28 March 2008
Received in revised form 3 June 2008
Accepted 9 June 2008
Available online 21 June 2008

Keywords:
Biosensor
Piezoelectric membrane
Immunosensor array
Mass sensor

ABSTRACT

This paper reports a micro-machined piezoelectric membrane-based biosensor array for immunoassay. Goat immunoglobulin G (IgG) and HBsAg were immobilized as the probe molecules on the square piezoelectric membranes of the sensors that have dimensions of 3.5 μm × 500 μm × 500 μm. Due to the mass sensitive nature of these sensors, their resonant frequencies were depressed after the anti-goat IgG or anti-HBsAg was captured by the goat IgG or HBsAg. The resonant frequencies of the sensors were measured by an impedance analyzer. The experimental results demonstrate that the measured frequency change varies from 100 to 700 Hz, and the mass sensitivity of the device is estimated to be about 6.25 Hz/ng. A near linear relationship between the frequency change and the concentration of goat IgG was obtained, and the mass of the attached anti-goat IgG was calculated. The preliminary results discussed in this work indicate that the micro-machined piezoelectric membrane-based biosensor has a potential application as an immunosensor.

1. Introduction

A biosensor generally consists of a transducer and an affinity-based interface, which binds the biological receptors, and generates a detectable signal when the interested biological entities are captured. The transducer serves as sensor platform to detect the signal generated by the interface (Li et al., 2004).

Micro-machined piezoelectric biosensors are being widely investigated due to their many advantages over other sensors, such as compact size, high sensitivity, easy integration with an analysis circuit, and rapid response (Janshoff et al., 2000). Depending on the sensing platform of the sensor, micro-machined piezoelectric biosensors can mainly be classified into cantilever type, quartz-crystal micro-balance (QCM) type, and membrane type.

To date, micro-machined piezoelectric cantilever-based sensors (MPCS) are still the major player in the field of biosensing because of their high sensitivity and label free detection (Raiteri et al., 2001; Carrascosa et al., 2006). Most of these sensors use the mass micro-balancing technique to measure the change in resonant frequency after the capture of target molecules on a functionalized micro-cantilever surface (Hwang et al., 2004; Lee et al., 2006; Kang et al., 2006). However, MPCS suffers from a low quality merit factor (*Q* value), which makes it very difficult to be used in a liquid media. Furthermore, the fragility of these devices during fabrication processes and under real operating conditions also limits their extensive applications.

To overcome this issue, the QCM is developed as an alternative. Quartz has been successfully used in high sensitivity gas and liquid sensors for more than 30 years. Recently, QCM was reported to have successfully detected DNA and bacteria with good frequency stability and reproducibility (Mo et al., 2002; Mannelli et al., 2003). While this is a positive advancement, QCM remains unpractical for miniaturization and biosensor array application.

An interesting device based on micro-machined resonating piezoelectric membrane arrays was recently reported (Nicu et al., 2005). This device avoids problems, such as the fragility of the silicon-based cantilever in the micro-cantilever sensor and the lack of integration potential in the QCM biosensors. However, the affinity-based interface of this device is the open top surface of the membrane, and the reactive liquid that contains biomaterial can flow freely from one sensor to another sensor, resulting in undesired interaction between different biomaterials. Thus, this sensor array is not suitable for multi-biomaterial detection.

In this paper, we present another piezoelectric membrane-based biosensor array, which is fabricated with micro-machining techniques. This new device has a very compact size, relatively high sensitivity, rapid response, and it can be integrated with an analytical circuit easily. Additionally, this sensor array can detect multiple biological materials simultaneously because the interactions between the biomaterials take place within each reaction chamber. After characterization, it was verified that this sensor array can be used as an immunosensor. The working principle,

* Corresponding author. Tel.: +65 67906038; fax: +65 67924062.
E-mail address: mjmmiao@ntu.edu.sg (J. Miao).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.06.024

fabrication and characterization results will be introduced in the following section.

2. Mass sensitivity—theoretical analysis

The piezoelectric membrane-based biosensor presented here actually worked as a mass sensor. The working principle of this sensor is that the resonant frequency of the membrane decreases in response to a mass loading on the membrane surface. For an amount of mass added onto the micro-machined membrane surface, the resonant frequency change of the sensor is proportional to the added mass. The relationship is shown as follows:

$$\Delta f \propto \Delta m \tag{1}$$

where Δf denotes the frequency change, and Δm stands for the amount of mass added onto the device. The mass sensitivity S_M of a piezoelectric mass sensor can be defined as (Wenzel and White, 1989)

$$S_M = \lim_{\Delta M \to 0} \frac{\Delta f}{f_0} \frac{1}{\Delta M} \tag{2}$$

where ΔM is the mass per unit area loaded onto the surface of the sensor, and $\Delta f = f - f_0$ is the frequency change in response to the mass loading. The unit of S_M is m^2/kg. The resonant frequency of a thin square membrane with side width a can be expressed as

$$f_0 = \frac{\lambda_{ij}^2}{2\pi a^2} \sqrt{\frac{D}{\rho d}} = \frac{\lambda_{ij}^2}{2\pi a^2} \sqrt{\frac{D}{M}} \tag{3}$$

where $D = Ed^3/12(1-\nu^2)$ is the flexural rigidity, λ is a constant, ρ and d are the density and thickness of the membrane, respectively, and $M = \rho d$ is the mass per unit area of the membrane. Differentiating (3) with respect to M and using the definition (2), the mass sensitivity can be calculated as

$$S_M = \lim_{\Delta M \to 0} \frac{\Delta f}{f_0} \frac{1}{\Delta M} = -\frac{1}{2M} = -\frac{1}{2} \frac{1}{\sum_i \rho_i d_i} \tag{4}$$

where $M = \sum \rho_i d_i$ for the case of a multilayer membrane, and ρ_i and d_i are the density and thickness of the i^{th} layer of the membrane, respectively.

The fabricated membrane is simplified as a 3.5-μm-thick piezoelectric layer on a 1.8-μm-thick silicon oxide layer during the theoretical calculation. The densities of $Pb(Zr_{0.52}Ti_{0.48})O_3$ (PZT) and SiO_2 layers used for the simulation are 7.62×10^3 kg/m^3 and 2.07×10^3 kg/m^3, respectively (Yao et al., 2003). Based on Eq. (4), the theoretical mass sensitivity (S_M) of the fabricated sensor is calculated to be -16.5 m^2/kg.

An alternate definition of mass sensitivity is the resonant frequency change corresponding to a unit mass load (Thundat et al., 1995; Ilic et al., 2000). It can be written as

$$S_m = -\frac{\Delta f}{\Delta m} \tag{5}$$

where Δf is the frequency change due to an external introduced mass load Δm. The unit of S_m is Hz/kg. A larger absolute value of S_m means that the device is more sensitive.

Referring to Eqs. (3) and (4), the relationship between S_m and S_M can be rewritten as

$$S_m = -\frac{\Delta f}{\Delta m} = -\frac{f_0}{A} S_M \tag{6}$$

where f_0 is the resonant frequency before any mass is added to the membrane, S_M is the sensitivity in units of m^2/kg, and A is the area of the membrane. Since the dimensions and material properties of the membrane are constant, Eqs. (4) and (6) reveal that the sensitivity is already defined once the sensor is fabricated. The mass change is proportional to the detected frequency change and can be easily derived from Eq. (6) as

$$\Delta m = \frac{A}{S_M f_0} \Delta f \tag{7}$$

where f_0 is the resonance frequency which can be determined using impedance analysis due to the piezoelectric effect. S_M can be obtained from Eq. (4). Therefore, the mass of the biological entity captured by the bioreceptor is proportional to the resonance frequency change of the membrane, and thus can be calculated by measuring the resonant frequency change. This result is quite significant in quantitative mass analysis using such a sensor.

3. Micro-fabrication of the sensor array

3.1. Fabrication processes

The piezoelectric membrane-based biosensor array was fabricated using standard bulk micro-machining fabrication techniques. There were a total of 10 main steps involved. (1) A thermal silicon oxide layer (1.8 μm thick) was grown on a 4-in. double-sided polished silicon wafer. (2) A silicon nitride layer with a thickness of 200 nm and a lower temperature oxide (LTO) layer with a thickness of 350 nm were then deposited by low pressure chemical vapor deposition (LPCVD) on both sides of the wafer. (3) To open a window for backside silicon etching, the LTO and Si_3N_4/SiO_2 layers were etched by buffered oxide etching (BOE) and reactive ion etching (RIE). (4) After patterning, KOH wet etching was performed until the remaining thickness of silicon was about 50 μm. (5) Ti (20 nm)/Pt (200 nm) layers were sputtered, patterned on front side and used as bottom electrode. (6) PZT was used as the piezoelectric material for the membrane. A thick PZT layer with a required thickness of around 3.5 μm was deposited by the composite thick film deposition technique (Zhu et al., 2002; Wang et al., 2003). (7) Wet etching of the PZT in diluted HCl/HF solution was done to expose the bottom electrode pad. (8) A polyimide layer was spin-coated, patterned, and cured as an insulation layer to minimize parasitic capacitance induced by the patterned electrode wiring. (9) Ti (20 nm)/Pt (200 nm) layers were sputtered and patterned on the front side using lift-off to serve as the top electrode. (10) The backside silicon was etched away by KOH till the SiO_2 layer was exposed. (11) A layer of Au with thickness of 50 nm was sputtered on the surface of SiO_2 as the immobilization surface of the reaction chamber.

3.2. Fabrication results

Using the micro-machining process described above, the membrane-based piezoelectric biosensor array was successfully fabricated. As shown in Fig. 1(a), each array consists of seven individual sensors which are located in a hexagonal shape. Each square block shown in Fig. 1(a) is actually a single sensor. The fabrication of the device could obtain a very high yield due to the high quality thick PZT layer.

The backside of the array, which shows the reaction chambers, is illustrated in Fig. 1(b). The width of the sensor was about 0.51 mm, which was close to the designed value (0.5 mm). The opening area of the chamber was much larger than that of the bottom membrane due to the anisotropic wet etching by KOH. The subsequent biomaterial immobilizations processes, such as the dipping and washing of the biomaterial, were much more convenient due to this enlarged opening area.