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A new device for liver cancer biomarker detection with high accuracy

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ABSTRACT

A novel cantilever array-based bio-sensor was batch-fabricated with IC compatible MEMS technology for precise liver cancer bio-marker detection. A micro-cavity was designed in the free end of the cantilever for local antibody-immobilization, thus adsorption of the cancer biomarker is localized in the micro-cavity, and the adsorption-induced k variation can be dramatically reduced with comparison to that caused by adsorption of the whole lever. The cantilever is pizeoelectrically driven into vibration which is pizeoresistively sensed by Wheatstone bridge. These structural features offer several advantages: high sensitivity, high throughput, high mass detection accuracy, and small volume. In addition, an analytical model has been established to eliminate the effect of adsorption-induced lever stiffness change and has been applied to precise mass detection of cancer biomarker AFP, the detected AFP antigen mass (7.6 pg/ml) is quite close to the calculated one (5.5 pg/ml), two orders of magnitude better than the value by the fully antibody-immobilized cantilever sensor. These approaches will promote real application of the cantilever sensors in early diagnosis of cancer.

1. Introduction

Recently, MEMS-based technologies are playing more and more important roles in early diagnosis of cancer due to their high sensitivity, fast response, low cost, small reagent consumption, portability, real-time, label-free detection, and so on. Several methods for detecting serum biomarkers have been developed, for example, surface plasmon resonance (SPR), quartz crystal microbalance (QCM), and micro-cantilevers, etc [1]. Among them, cantilever sensors are the most attractive candidate for practical application in early diagnosis of cancer from the viewpoint of batch-fabrication, cost, and reliability [2–5].

For the cantilever sensor working in a dynamic mode, the resonance frequency shift depends on variation of mass m and lever stiffness k. However, in conventional cantilever sensors working on mass-loading principle, the effect of k change is neglected, this results in distinct error for mass detection [6]. Lee J. H. found that the measured frequency change (184 Hz) is two orders of magnitude larger than the theoretical value according to mass loading of C-reactive protein (2 Hz) [7]. Similar results was reported by Gupta A. K., the calculated frequency change (24 kHz) is 5 times smaller than the experimental value (132 kHz), and it was experimentally demonstrated that the frequency change depended crucially on the attachment kinetics of biomolecules on the cantilever [8].

Adsorption-induced surface stress made substantial contribution to the detection error. Both theoretical and experimental studies have been undertaken to investigate the effect of surface stress in microcantilevers. Hwang et al. reported that molecular interactions generate surface stress on the microcantilever surface and modify the lever stiffness, but it is difficult to distinguish the frequency shift caused by mass loading and the stiffness variation from the measured frequency change of 100–600 Hz responding to the antigen concentrations of 10 and 100 ng/ml [9]. Wang et al. studied the effect of gas adsorption-induced surface stress on mechanical properties of ultra-thin silicon cantilever with different orientations, but no quantitative description on the adsorption-induced stress is included [10]. Nevertheless, until now, there is no feasible method to quantitatively estimate the effect of surface stress on the accuracy for mass detection, although it is essential for practical application of cantilever-based biosensors in mass detection with high sensitivity.

In this paper, a novel cantilever arrays sensor is batch-fabricated for precise bio-marker detection. A local biochemical reaction cavity is designed in the free end of the cantilever to reduce the effect of k variation with adsorption. And an analytical model integrating the adsorption-induced surface stress has been established to eliminate the effect of k change on mass detection

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accuracy. These modifications have dramatically improved the performance of the fabricated biosensor, which is capable of sensing the liver cancer biomarker-AFP with high accuracy.

2. Material and method

2.1. Fabrication

Fig. 1 shows the illustration of the device. Cantilever arrays are designed to jointly detect multi-biomarkers. The cantilever is driven by piezo and the response signal is piezoresistively sensed and detected by Wheatstone bridge circuits. The reference cantilever is designed to eliminate the influence of non-specific adsorption and environment noise. In order to reduce the effect of the adsorption-induced k change, the micro-cavity are designed in the free end of the cantilever to serve for local interaction with the antigen. The pillar arrays in the micro-cavity are made for increasing detection upper limit. These structural features offer several advantages: high mass detection accuracy, high throughput, small volume for portable system.

The device is batch fabricated with CMOS compatible processes. The ⟨100⟩ oriented n-type silicon top layer of 4-inch SOI wafer has a thickness of 5 μm. Fig. 2 gives the schematic of fabrication processes. Firstly, a 200 nm thick SiO_2 layer is grown by dry oxidation to serve as the mask for ion implantation, and an additional SiO_2 mask layer is deposited by plasma enhanced chemical-vapor deposition (PECVD) on the backside for deep etching in step (a). Then boron implantation is done for making the piezoresistors and the electrical lines, and is followed by annealing at 1000 °C. Next, the top Si layer is etched by reactive ion etching (RIE) to define the local reaction cavity in step (b). In step (c), the cantilevers are shaped by RIE. Lift-off is done to make the Cr/Au electrodes in step (d) and a PECVD SiO_2 mask is made on the front side of the wafer to protect the cantilevers in step (e). Subsequently the Si substrate is etched to define the chip body from the backside by deep reactive ion etching (DRIE) until a desired depth, and an isotropy RIE is employed to remove the residual Si substrate from the front side of the wafer. Finally, the cantilevers are released by BOE in step (f). The SEM pictures of the device are shown in Fig. 3.

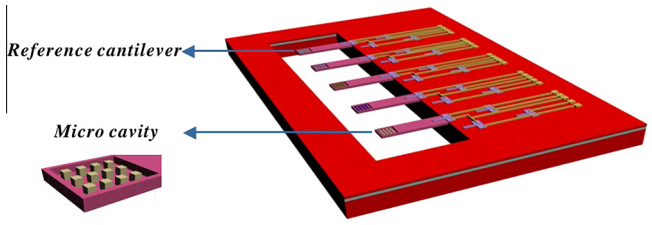

Fig. 1. Illustration of the cantilever arrays, the micro-cavity on the free end of the cantilever with pillar arrays.

2.2. Functionalization of the cantilever

The functionalization process of the cantilever and the locally immobilized lever with AFP antibody are depicted in Fig. 4(a) and (b). The silicon cantilever is oxidized using oxygen plasma and subsequently silanized at 24 °C using a 10% 3-aminopropyltriethoxysilane (APTES) solution in ethanol for 1 h. Freshly silanized-cantilever is incubated at 24 °C in 5% glutaraldehyde GA solution for 1 h to form a stable bond between $–NH_2$ and –CHO and then washed in phosphate buffered saline (PBS, pH 7.4), thus the cantilever is able to bind with protein (three times washing in buffer is done for all the cantilevers). Then the fluorescent Cy-5 labeled immunoglobulin G (IgG) antibody at a concentration of 40 μg/mL is locally immobilized in the micro-cavity of the lever by micro printing technology and incubated for 1 h and whereafter the levers are washed in PBS, as shown in Fig. 4(b). The micro printing system can print various antibodies on different cantilever at wafer level to ensure simultaneous detection of multiple biomarkers. The other active site of the cantilever is terminated by bovine serum albumin (BSA) solution at 24 °C over night, thus only antibody-bounded micro-cavity could capture the AFP antigen. For comparison, the IgG antibody was immobilized on the whole surface of some cantilevers. IgG is purchased from the company of Thermo (Massachusetts, USA).

The mechanical properties of the cantilevers were characterized by laser Doppler vibration system. Fig. 5 is a typical resonance spectrum of the cantilever. The quality factors (Q) of the cantilever are 587 in air and 49,434 in vacuum, good enough for achieving high sensitivity. For the cantilever of l = 195 μm, w = 75 μm, and h = 5 μm, the sensitivity and the mass resolution are 0.31 pg/Hz and 0.075 pg, respectively, high enough for early diagnosis of cancer.

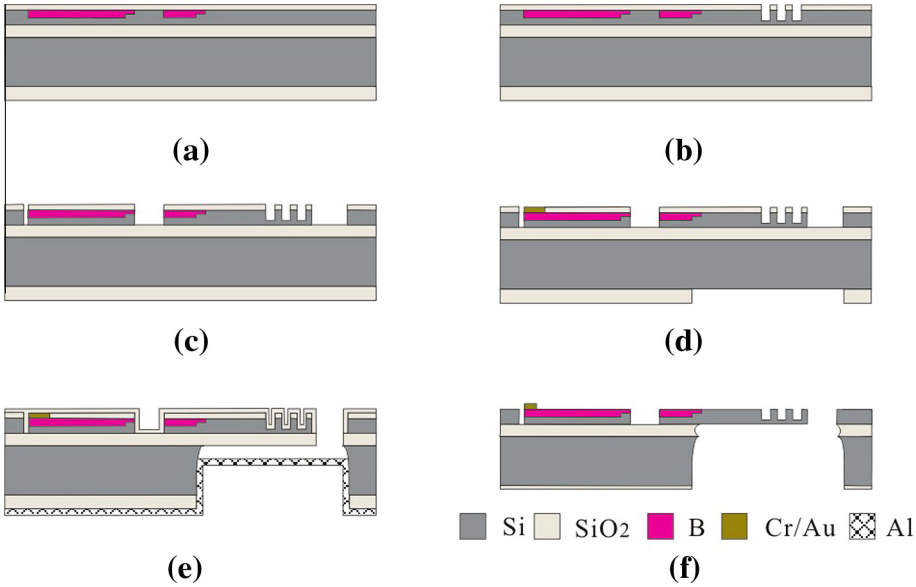

Fig. 2. Fabrication process flow of the cantilever.