Sensors and Actuators B 243 (2017) 775–783

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Comparative analysis of static and non-static assays for biochemical sensing using on-chip integrated field effect transistors and solidly mounted resonators

Wenpeng Liu, Hao Zhang, Hongyuan Zhao, Zifan Tang, Yanyan Wang, Chongling Sun, Wei Pang, Xuexin Duan*

State Key Laboratory of Precision Measuring Technology & Instruments, College of Precision Instrument and Opto-electronics Engineering, Tianjin University, Tianjin 300072, China

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form 6 December 2016
Accepted 12 December 2016
Available online 13 December 2016

Keywords:
Micro/nano biochemical sensor
Extended-gate field effect transistor (EGFET)
Solidly mounted resonator (SMR)
Static assay
Non-static assay

ABSTRACT

The advancement in micro/nanotechnologies has been continuously providing possibilities for inventing novel biochemical sensors. However, variations in the transducer type can cause different sensing results due to the differences in their mechanisms of analyzing biomolecular interactions. In this work, we focused on the comparative analysis of static and non-static assays for molecular interactions using on-chip integrated extended-gate field effect transistor (EGFET) as a static sensing interface and solidly mounted resonator (SMR) as a non-static sensing interface. Analysis of polyelectrolytes (PETs) surface assembly and antigen-antibody interaction using the two types of biochemical sensors presented consistent and complementary sets of information. Meanwhile, due to the difference in their operating mechanisms, variations on the detection efficiency, kinetics and thermodynamics were observed. Our results highlighted the critical dependence of signal detection on biochemical sensors' operating mechanisms and provided a valuable guidance for static and non-static assays for biomolecular detections.

1. Introduction

Analysis of biomolecular interactions such as antigen-antibody binding, protein-ligand interactions and enzyme-substrate interactions plays a vital role in biochemical field ranging from medical diagnostics and clinical therapy to environmental monitoring and catalyst engineering [1–5]. These applications instigated the development of micro/nanobiochemical sensors possessing great advantages including low sample amounts, low power consumption, high sensitivity, high portability, and its applicability in multiplexed detections [3,6,7]. One of the recent trends in developing micro/nanobiochemical sensor platform is the design and fabrication of on-chip integrated biochemical sensor arrays containing two or more different types of transducers [3,8]. Various types of micro/nanotransducers such as optical [7], electrochemical [9], electromechanical [10], resistive [11], and capacitive devices [12], have been proposed for the development of on-chip integrated biochemical sensors. With the advancement of the micro/nanofabrication and sensing technologies, many challenges were opened in the field of on-chip integrated multiplexed biochemical sensor arrays such as the issues of sensitivity, selectivity, reliability, miniaturization and device crosstalk.

Biochemical sensors can be classified into two types based on their operating mechanism—(1) the static biochemical sensor which uses a static sensing interface and (2) the non-static biochemical sensor containing a non-static sensing interface. In this work, we analyzed the biomolecular interactions between the antibody and antigen using both static and non-static sensors, highlighting the critical dependence of the sensing results on the operating mechanisms of the transducers. To achieve this, we designed and fabricated an on-chip integrated microsystem consisting of field effect transistor (FET) (static assay) and solidly mounted resonator (SMR) (non-static assay) for simultaneous detections of biomolecular interactions (Fig. 1). Both transducers satisfy the requirements of availability and affordability for massive production through standard wafer-scale semiconductor fabrications. The most important feature of this on-chip integrated multiplexed microsystem is its ability to carry out the simultaneous analysis of biomolecular interactions from different physical parameters. We comparatively analyzed the responses of charge-sensitive FET and mass-sensitive SMR for the detection of polyelectrolytes (PETs) surface assembly in a layer-by-layer

* Corresponding author.
E-mail address: xduan@tju.edu.cn (X. Duan).

http://dx.doi.org/10.1016/j.snb.2016.12.065
0925-4005/

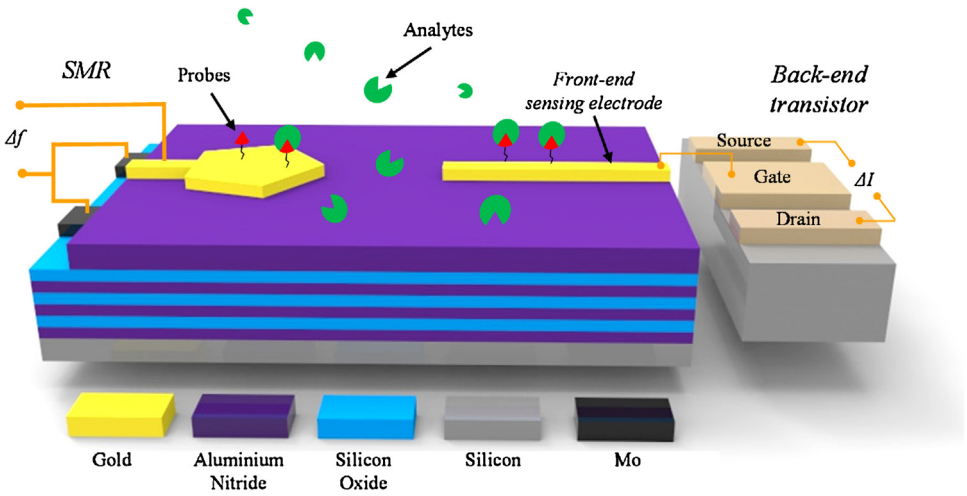

Fig. 1. Diagram depicting the configuration of on-chip integrated EGFET and SMR for simultaneous monitoring of molecular interactions.

(LbL) fashion. Antigen-antibody binding interaction was analyzed as well to further investigate the impact of operating mechanisms on biosensing results.

2. Materials and methods

2.1. Reagents and materials

Poly(allylamine hydrochloride) (PAH) (M_w = 58000) and poly(styrene sulfonate) (PSS) (M_w = 70000) were purchased from Sigma. 16-mercaptohexadecanoic acid (MHDA), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Aladdin Industrial Corporation. 5 μm (diameter) polystyrene (PS) particle suspension (Aladdin Industrial Corporation) was introduced for flow characterization. Mouse monoclonal anti-human prostate specific antigen (anti-PSA) and native human prostate specific antigen (PSA) were purchased from Linc-Bio Science Co. LTD (Shanghai, China). All chemicals were used as received without further purifications.

2.2. Chip fabrication

Bragg reflector was first fabricated by alternately depositing three pairs of aluminum nitride (AlN) and silicon dioxide (SiO_2) thin films on silicon wafer followed by 600 nm molybdenum (Mo) growth as the bottom electrode. Then, the highly c-axis oriented 1 μm AlN film acting as piezoelectric layer was deposited by radio frequency (RF) reactive magnetron sputtering. Finally, 300 nm Au was employed as the top electrode for SMR as well as the extended gate (EG) for FET. To demonstrate the ability of high-throughput and multiple detections, as well as selective functionalization on the specific sensor [13], an array consisting four pairs of EG and SMR was fabricated on a 1 cm × 1 cm chip.

2.3. Measurement setup

The chip was mounted onto a four-channel evaluation board specifically designed for RF measurement. SMR was wire-bonded to the sub-miniature-A (SMA) connector and then connected to a PC-controlled vector network analyzer (VNA, B5071C, Keysight). EG was wire-bonded to a commercially available back-end transistor (ALD110800A, Advanced Linear Devices). The back-end transistor was an n-channel FET with a zero-volt threshold voltage necessary to avoid the destruction of biomolecules at any positive or negative solution voltage. A miniature Ag/AgCl reference electrode (World Precision Instruments, Inc.) was used to bias the transistor to the desired working point. Characteristic of transistor was confirmed off-line using Keithley 2636 and 2400 Source Measure Unit before real-time measurement at a constant drain-source voltage (V_{ds}=100 mV). This will serve as a look-up table to convert the measured drain current (I_d) back into the interfacial potential (V_g) of sensing electrode [14], Electrochemical impedance spectroscopy (EIS) measurements were performed using an electrochemical workstation (Princeton Applied Research, VersaSTAT 4). Pt and Ag/AgCl were used as the counter electrode (CE) and reference electrode (RE), respectively. Micro particle image velocimetry (micro-PIV) was used to characterize the microvortices. PS particles were tracked using a video camera (Olympus DP73, Tokyo, Japan) attached to an optical microscope (Olympus BX53, Tokyo, Japan) and their movement were analyzed with Diatrack 3.04. The three-dimensional distribution of PETs molecules under the actuation of SMR was measured using a homemade reflection digital image-plane holographic microscopy (DIPHM). The illumination source was a tunable diode laser working at λ = 690 nm (Nanobase, Xperay-TL-STD, 639–697 nm) and was split into the object beam and the reference beam. The expanded object beam illuminated and reflected from SMR surface to create the object wave front, which interfered with the reference wave front, forming the surface profile in 3D perspective images.

2.4. PETs surface assembly and antigen-antibody binding

PAH and PSS solutions (1 mg/ml) were prepared by dissolving PAH and PSS separately into 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) with 50 mM NaCl (pH = 6). The solutions were sonicated for 15 min, followed by overnight incubation at room temperature to get uniform solutions. Before PETs surface assembly, the chip was cleaned by immersion in piranha solution for 15 s. The chip was rinsed with substantial amounts of ultrapure water. The cleaned chip was then wire-bonded to the four-channel evaluation board. A reservoir with input and output fluidic channels was created by gluing a polypropylene tube onto the surface of the chip to hold the solution. The exposed wire bonds were sealed with epoxy to protect them from ions existing in the air. PETs solutions were