Biosensors and Bioelectronics 26 (2010) 1565–1570

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Integration of microfluidic and cantilever technology for biosensing application in liquid environment☆

Carlo Ricciardi[a,*], Giancarlo Canavese[b], Riccardo Castagna[a], Ivan Ferrante[a], Alessandro Ricci[a], Simone Luigi Marasso[a], Lucia Napione[c], Federico Bussolino[c]

[a] LATEMAR – Politecnico di Torino, Dipartimento di Scienza dei Materiali ed Ingegneria Chimica, Corso Duca degli Abruzzi 24, I-10129 Torino, Italy
[b] IIT – Italian Institute of Technology @ POLITO Center for Human Space Robotics, Corso Trieste 21, 10129 Torino, Italy
[c] Department of Oncological Sciences, Institute for Cancer Research and Treatment, University of Torino, 10060 Candiolo (TO), Italy

ARTICLE INFO

Article history:
Received 14 April 2010
Received in revised form 9 July 2010
Accepted 29 July 2010
Available online 5 August 2010

Keywords:
Lab-on-Chip
Microcantilever
Real-time
Protein detection
Angiogenesis

ABSTRACT

Microcantilever based oscillators have shown the possibility of highly sensitive label-free detection by allowing the transduction of a target mass into a resonant frequency shift. Most of such measurements were performed in air or vacuum environment, since immersion in liquid dramatically deteriorates the mechanical response of the sensor. Besides, the integration of microcantilever detection in a microfluidic platform appears a highly performing technological solution to exploit real time monitoring of biomolecular interactions, while limiting sample handling and promoting portability and automation of routine diagnostic tests (Point-Of-Care devices). In the present paper, we report on the realization and optimization of a microcantilever-based Lab-on-Chip, showing that microplates rather than microbeams exhibit largest mass sensitivity in liquid, while pirex rather than polymers represents the best choice for microfluidic channels. Maximum Q factor achieved was 140 (for fifth resonance mode of Pirex prototype), as our knowledge the highest value reported in literature for cantilever biosensors resonating in liquid environment without electronic feedback. Then, we proved the successfully detection of Angiopoietin-1 (a putative marker in tumor progression), showing that the related frequency shifts coming from non-specific interactions (negative controls) are roughly one order of magnitude lower than typical variations due to specific protein binding. Furthermore, we monitored the formation of antibody–antigen complex on MC surface in real-time. The proposed tool could be extremely useful for the comprehension of complex biological systems such as angiogenic machinery and cancer progression.

1. Introduction

Resonance operation method, which aims to quantify the adsorbed target mass thanks to the oscillator frequency shift, seems to be the most successful application of microcantilever (MC) based biosensor. Despite of static operation method (where surface stress generated in binding of the target molecules to the receptors on one MC side cause the beam to deflect), this technique is less affected by the thermal drift of beam deflection and stabilization problems (Shen et al., 2001; Lochon et al., 2006).

The progress of microcantilever technology and the need for increasing device sensitivity have favored the reduction of sensor dimensions up to the nanoscale. It has been shown that nanocantilevers are able to detect few biomolecules or single viruses (Ilic et al., 2004; Gupta et al., 2004), thus displaying very high mass sensitivity. On the other hand, biosensors at the nanoscale have recently shown evident performance limits in terms of analyte density, response time (Nair and Alam, 2006) and statistical variability (Gupta et al., 2006) due to their intrinsic diffusion-limited regime. Furthermore, such highly sensitive can be achieved in air or vacuum environment, since immersion in liquid would dramatically deteriorate the response of the sensor. Indeed, minimum detectable mass can be defined as $\Delta m_{\min} \propto (m/Q)$ (Waggoner and Craighead, 2007), where m and Q are the oscillator mass and quality factor, respectively. The latter is defined as $Q = f_r(\Delta f_{-3\,\mathrm{dB}})^{-1}$, where f_r is the resonance frequency and $\Delta f_{-3\,\mathrm{dB}}$ is the width of the resonance curve at -3 dB from the maximum.

Operation in liquid in fact would be desirable in order to retain biomolecule physiological structure and function, since it has been shown that proteins and cell membrane can change their conformation passing from liquid environment to vacuum condition (Sharma and Kalonia, 2004). Liquid environment is then fundamental in those applications where the observation of binding and unbinding kinetics are needed as well as for in situ and real-time measurement. An on-line measurement in liquid can also reduce false positive and false negative responses, which are an

☆ Finalist for the World Congress on Biosensors 2010 selected Keynote Paper.

* Corresponding author. Tel.: +39 011 0907383.
E-mail address: carlo.ricciardi@polito.it (C. Ricciardi).

0956-5663/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2010.07.114

important drawback in MC-based biosensing (Waggoner and Craighead, 2007). Moreover, operation in liquid assures the reduction of the risk of breaking the MCs in transporting them between different environments as well as during the drying procedure. Finally, in vacuum conditions, residual salt precipitated from the buffer solution, employed in cleaning or functionalization steps, could remain on cantilever surfaces thus invalidating target mass estimation (Liu et al., 2009).

Recent literature contains some interesting studies about cantilevers vibrating in liquid performed under static fluid conditions (Campbell and Mutharasan, 2005a; Park et al., 2005). Campbell and Mutharasan (2005b) performed measurements using millimeter-sized cantilever partially immersed in a static liquid; this approach has the disadvantage to register a decrease of volume during the experiment due to the evaporation and consequently it requires a more complex calibration. It is worth to note that almost all the experiments concerning cantilevers immersed in liquid are carried out in commercial or home-made fluid cell (Arntz et al., 2003; Braun et al., 2005; Ghatkesar et al., 2008; Moulin et al., 2000; Vančura et al., 2005) with volume from tens of microliters to 2 mL, in which the device is placed and sealed through o-ring or rubber membrane. Maraldo et al. (2007a, 2007b) and Campbell et al. (2007) obtained great results in biomolecules mass detection at different concentrations by piezoelectric-excited millimeter-sized cantilever (PEMC). Since the cantilevers are made individually and manually, a low reproducibility and repeatability in fabrication process is obtained. In addition their design is difficult to be integrated in standard low cost production both for what concerns the materials and the processes. The large dimension of the PEMC forces to design a wide housing flow cell and the fabrication processes are not easy to integrate in Lab-on-Chip (LOC) technology.

Very few works report a fluidic circuit integrated on microcantilever sensor device (Park et al., 2008; Thaysen et al., 2001). Aubin et al. (2005) presented a design of integration between resonator sensor array and microfluidic channel using micromachining processes. Since the dimensions of their resonators are about 10 μm, during the measurements, the microfluidic channels hosting the sensors are pumped down to a pressure where damping effects become negligible. This last solution appears to be expensive and does not promote integration and device portability.

It is clear, therefore, that MC biosensors would benefit in case of integration with microfluidic technology; advantages such as reduction of volume reagents and time assay, in situ measurements and high level of automation are evident. Moreover, diagnostic biosensing protocols, as well as single-molecule detection, could express their practical potentiality only with microfluidic assembly, where total fluidic volumes is minimized, recirculation procedure can be created to achieve enhanced binding probabilities, and the environment to which the sensor is exposed can be actively controlled.

In this paper, we report on the realization and optimization of a MC-based LOC to perform in liquid real-time detection of specific proteins. We chose Angiopoietin-1 (Ang-1) as our target molecule representative of the wide range of angiogenic factors, whose expression level is largely investigated in different tumors. Angiopoietin-1 and -2 are oligomeric-secreted glycoprotein ligands of the receptor tyrosine kinase Tie-2 (Davis et al., 1996). Even if it is well established that the Ang–Tie-2 pathway is involved in tumor angiogenesis, the exact effects of angiopoietins on tumor angiogenesis are under debate (Metheny-Barlow and Li, 2003). Nevertheless experimental and clinical studies have demonstrated that increased expression of Ang-1 and -2 promotes or inhibits tumor angiogenesis (Yu, 2005), suggesting that Ang-1 is a pro-angiogenic factor that promotes endothelial cell survival and tumor angiogenesis, especially in the presence of vascular endothelial growth factor.

Four main objectives can be identified in the following work: (I) optimization of sensor geometry (in particular, MC aspect ratio); (II) optimization of microfluidic platform (in particular, best choice for materials of microchannels and interconnections); (III) successfully in liquid detection of Ang-1 (with respect to negative controls); (IV) observation of antibody–antigen real-time kinetics.

2. Materials and methods

2.1. Reagents

3-Aminopropyltriehoxysilane (APTES, anhydrous, 99% Aldrich), glutaraldehyde (GA, 25%, v/v, water solution) and toluene (anhydrous, 99.8% Aldrich) were used without any further purification. Sulphuric acid (95–97%, w/w) and hydrogen peroxide (30%, w/w) were also purchased from Sigma–Aldrich. Orthoboric acid and sodium chloride used to prepare borate buffer were ACS reagents (assay ≥ 99.5%) and were obtained from Sigma–Aldrich. Recombinant Protein G, purified from Streptococcus, was from PIERCE. Ang-1 was obtained from R&D Systems, while the anti-Ang-1 (A0604) mAb was from SIGMA. Dulbecco's Phosphate-Buffered Saline (PBS; BE17-512F) was procured from BioWhittaker/Cambrex.

2.2. LOC fabrication

The cantilever chips were fabricated starting from Silicon On Insulator (SOI) wafers with the following layers and thicknesses: polished silicon device layer of 7 ± 0.5 μm, silicon handle layer of 450 ± 5 μm, buried and backside oxide layers 1.5 ± 0.1 μm. The process flow is composed of the following steps: photolithography on the back side, wet etching in Buffered Oxide Etch (BOE) solution for the patterning of the mask layer on the back side, front side protection with a polymeric coating (Protek B2 from Brewer Science), wet etching in KOH solution, sample cleaning, photolithography on the front side of the membrane, Reactive Ion Etching (RIE) of silicon, removal of the buried oxide layer in BOE and cleaning by piranha solution (Canavese et al., 2007). The complete process flow, together with additional MC fabrication details, is reported in Supplementary material.

Three different materials (SU-8, PDMS, Pirex) and relative process fabrication were chosen for three different microfluidic prototypes of LOC for the integration with microcantilever sensors. All layouts have at least two wells (roughly 2 μL in volume, each), in order to perform different biodetection experiments simultaneously. Polydimethylsiloxane (PDMS) was selected for the fabrication of the microfluidic interconnection for its peculiar characteristics as biocompatibility, transparency, cost-efficiency, etc. A particular interconnection layout requiring no external clamping or gluing was studied. For all the prototypes were adopted the same PDMS microfluidic reversible interconnections designed and fabricated as described in a previous study (Quaglio et al., 2008).

A picture and a 3D sketch of the device, together with important parts of the experimental set-up are reported in Fig. 1a and b, respectively.

Processes flow description as well as fabrication details are reported in Supplementary material.

2.3. Experimental set-up and data analysis

Cantilever vibrational characteristics were measured using an apparatus in which a function generator (HP 33120A) produced a sinusoidal signal that was amplified and sent to a piezoelectric actuator (PI Ceramic). Cantilever resonance curves were monitored using an optical lever read-out (laser diode and PSD by Hamamatsu): the current output of the PSD was amplified and converted