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Multiplexed specific label-free detection of NCI-H358 lung cancer cell line lysates with silicon based photonic crystal microcavity biosensors

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ABSTRACT

We experimentally demonstrate label-free photonic crystal (PC) microcavity biosensors in silicon-on-insulator (SOI) to detect the epithelial–mesenchymal transition (EMT) transcription factor, ZEB1, in minute volumes of sample. Multiplexed specific detection of ZEB1 in lysates from NCI-H358 lung cancer cells down to an estimated concentration of 2 cells per micro-liter is demonstrated. L13 photonic crystal microcavities, coupled to W1 photonic crystal waveguides, are employed in which resonances show high Q in the bio-ambient phosphate buffered saline (PBS). When the sensor surface is derivatized with a specific antibody, the binding of the corresponding antigen from a complex whole-cell lysate generates a change in refractive index in the vicinity of the photonic crystal microcavity, leading to a change in the resonance wavelength of the resonance modes of the photonic crystal microcavity. The shift in the resonance wavelength reveals the presence of the antigen. The sensor cavity has a surface area of $\sim 11\ \mu m^2$. Multiplexed sensors permit simultaneous detection of many binding interactions with specific immobilized antibodies from the same bio-sample at the same instant of time. Specificity was demonstrated using a sandwich assay which further amplifies the detection sensitivity at low concentrations. The device represents a proof-of-concept demonstration of label-free, high throughput, multiplexed detection of cancer cells with specificity and sensitivity on a silicon chip platform.

1. Introduction

Detection of biomolecules on microarrays based upon label-free on-chip optical biosensors (Iqbal et al., 2010; Densmore et al., 2009; Sipova et al., 2010; Cunningham et al., 2004) is very attractive since this format avoids complex chemistries caused by steric hindrance of labels. In addition, chip-integrated technologies permit miniaturization contributing to the ultimate goal of personalized diagnostic assays for detecting toxins, allergens or biomarkers of disease, including cancer. Surface plasmon resonance (SPR) biosensors by Biacore (Sipova et al., 2010), and the Biomolecular Interaction Detector (BIND) platform (Cunningham et al., 2004) based on one-dimensional gratings in polymer substrates represent two technologies that have achieved commercial success. Devices based on two-dimensional photonic crystals in silicon (Lee and Fauchet, 2007; Zlatanovic et al., 2009; Lai et al., 2011, 2012; Chakravarty et al., 2012), have recently demonstrated the ability to confine and guide slow light on length scales of the wavelength of light leading to high sensitivity and miniaturization into compact sensors for chemical (Chakravarty et al., 2005) and bio-sensing (Lee and Fauchet, 2007; Zlatanovic et al., 2009; Lai et al., 2011, 2012; Chakravarty et al., 2012). A concern still remains regarding the specificity of the label-free interaction that is detected. Specificity can be established by a statistical determination based on the results from multiple sensor spots as well as via sandwich assays. We recently showed that multiple high sensitivity PC microcavity sensors can be arrayed on a chip and interrogated simultaneously by a single measurement. Using interconnecting on-chip waveguides, redundant measurements in multiple locations can be performed at the same instant of time (Zou et al., 2012). In this paper we present, to our knowledge, the first demonstration of multiplexed sandwich assay detection using the PC biosensor platform, combining simultaneous specific and control binding experiments for the detection of a biomarker from lung cancer cell lysates.

Primary lung cancer develops from epithelial cells lining the airways of the lung. Normally, these epithelial cells form a crucial barrier between the internal and external environments and in the lung, these cells prevent leakage of blood while assisting with

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exchange of O_2 and CO_2. Exposure to airborne particles and toxins, especially those found in cigarette smoke, leads to genetic changes in epithelial cells which accumulate and underlie progressive changes from hyperplasia to dysplasia to carcinoma in situ and frank cancer. As such tumors grow, they outstrip supplies of blood and oxygen, become stressed, and undergo the epithelial–mesenchymal transition (EMT), a process by which cells switch their epithelial gene expression patterns to a mesenchymal phenotype with increased migratory and invasive properties. This process is thought to underlie metastatic potential in many tumor types. A facile method for detection of the EMT state of tumor samples would have major importance both clinically and for basic science investigations. We and others have shown that ZEB1 and ZEB2 have a prominent role in controlling the EMT process in lung cancer (Gemmill et al., 2011; Takeyama et al., 2010). In this paper, we present proof-of-concept data that validate the ability of photonic crystal microcavity sensors to detect ZEB1 specifically via sandwich assays with high sensitivity.

2. Materials and methods

2.1. Photonic crystal fabrication.

The device is a photonic crystal (PC) microcavity coupled to a photonic crystal waveguide (PCW) in silicon on a silicon-on-insulator (SOI) substrate. The devices were fabricated using standard silicon wafer fabrication technologies in cleanroom facilities at the J.J. Pickle Research Center, Univ. of Texas, Austin. Precise methodologies for fabricating this type of device were described previously (Chakravarty et al., 2012).

2.2. Antibodies, coupling reagents and derivatization.

We coupled the following antibodies or proteins to the PC resonance cavities; anti-MYC 9E10 (Sigma Aldrich, Cat #: A3833 MYC-tag 9E10), anti-ZEB1 (H102, Santa Cruz, Cat #: sc-25388), pre-immune mouse IgG (BD Pharmingen™, Mat. #: 557273), bovine serum albumin (Invitrogen, Cat #: 15561-020). Chemicals including 3-aminopropyl-triethoxy-silane (3-APTES) (Acros, CAS #:919-30-2) and glutaraldehyde (Fischer Scientific, CAS#111-30-8) were used to functionalize the silicon surface using published procedures (Zou et al., 2012; Chakravarty et al., 2012; Subramanian et al., 1999). Devices were routinely washed three times in PBS before measurements and after each addition of target lysate.

2.3. Lung cancer cell line NCI-H358.

The lung cancer cell line NCI-H358 was obtained from the Tissue Culture Core facility of the Univ. of Colorado Cancer Center, Aurora, CO. It was stably transfected with a tetracycline-inducible 6-MYC-ZEB1 expression construct, as described by Gemmill et al. (2011).

3. Results and discussions

The PC waveguide (PCW) is a W1 line defect waveguide with uniform lattice constant $a=400$ nm, where W1 denotes that the width of the PCW is $\sqrt{3}a$. Silicon slab thickness and air hole diameter are $h=0.58a$ (232 nm) and $d=0.5775a$ (231 nm). Linear L13 PC microcavities with 13 missing holes along the Γ–K direction are fabricated two periods away from the PCW (Fig. 1A).

The edge air holes are shifted outward (Akahane et al., 2003) in the Γ–K direction by $0.15a$ (60 nm). A ~5–10 nm layer of silicon dioxide is functionalized to bind capture biomolecules to the device surface. For initial characterization, the silicon surfaces were functionalized and probe capture biomolecules were dispensed onto the PC microcavity. Details of the device simulation and fabrication have been published previously (Chakravarty et al., 2012).

Our PC microcavity sensor was designed considering that eventually the probe capture biomolecules would be dispensed by ink-jet printing. In ink-jet printing, the diameter of the ink-jet dispensed spot determines the minimum spacing between adjacent unique sensors, not the size of the sensor (Lai et al., 2012). This spot is approximately 35 μm and thus permits device densities as high as one every 50 μm.

Resonances in L13 PC microcavities such as the high quality factor (Q) ~26,760 in SOI structures (Lai et al., 2012) have been characterized previously. We experimentally demonstrated bio-sensing sensitivity down to 0.67 ng/ml, surface mass sensitivity of 0.8 pg/mm^2 on a sensing surface area of 11 μm^2, (Chakravarty et al., 2012) that compares extremely favorably with 1 pg/mm^2 sensitivity in SPR devices (Sipova et al., 2010).

Counter to current trends in photonic crystal sensor research, we designed PC microcavities slightly larger than the L3-type because of the physical advantages of higher Q possible in longer PC microcavities resulting from a larger mode volume with the combined effects of lower radiation loss and higher stored energy. In addition, our design achieves higher sensitivity because of the larger optical mode volume of the resonance cavity, a feature enabling more overlap with analytes that fill the 216 nm holes and coat the silicon surface. Highest sensitivity (Chakravarty et al., 2012) was achieved in silicon-on-insulator (SOI) structures at a biomolecule analyte concentration of 0.1 μg/ml.

A typical transmission spectrum of the W1 PCW device immersed in phosphate buffered saline (PBS) is shown in Fig. 1B. The coupled L13 PC microcavity device was covered with a representative probe capture antibody. The experimentally confirmed Q-factor in SOI is approximately 13,000, obtained as $\lambda/\Delta\lambda$ from the inset. In a multiplexed design, four L13 PC microcavities are arrayed on the four arms of a multimode interference (MMI) power splitter for simultaneous detection, as shown in Fig. 1C, (Zou et al., 2012).

In H358-ZEB1 lung cancer cells, expression of the ZEB1 gene was controlled with the tetraycycline derivative, doxycycline. Exogenous ZEB1 ($MW=\sim180$ kDa) was tagged at the N-terminus with 6 copies of the MYC epitope, permitting recognition by the anti-MYC antibody, 9E10. Samples were prepared from these cells prior to induction (0-day lysate) or after 3 days dox treatment to induce 6-MYC-ZEB1 (3-day lysate). Exogenous 6-MYC-ZEB1 present in the induced lysates can be detected using either the 9E10 antibody or an antibody that binds to native ZEB1 (Fig. 2).

This analysis verified that ZEB1 was strongly expressed in the dox-induced lysate (3d dox), was absent from the control lysate (0 dox), and that both antibodies are highly specific for ZEB1.

Anti-ZEB1 antibodies diluted 1:1000 in PBS and mixed with 35% glycerol in PBS were printed onto the functionalized PC microcavity and incubated overnight at 4 °C. After printing, the device was washed 3× with PBS, coated in BSA to block non-specific binding and re-washed 3× with PBS.

Devices were tested with TE-polarized light. Target lysates were introduced in PBS which forms the top cladding. When ZEB1-positive lysates bind to anti-ZEB1 antibodies attached to the PC microcavity, a change in the refractive index causes an alteration in the resonance frequency and a shift in wavelength of the dropped resonance from the PCW's transmission spectrum. The magnitude of the shift is precisely correlated with the concentration of ZEB1 in the lysates.