Biosensors and Bioelectronics 77 (2016) 315–322

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Small biomolecule immunosensing with plasmonic optical fiber grating sensor

Clotilde Ribaut [a,b,*], Valérie Voisin [b], Viera Malachovská [a,b], Valentin Dubois [a], Patrice Mégret [b], Ruddy Wattiez [a], Christophe Caucheteur [b,**]

[a] Proteomics and Microbiology Laboratory, University of Mons, Mons, Belgium
[b] Electromagnetism and Telecommunication Department, University of Mons, Mons, Belgium

ARTICLE INFO

Article history:
Received 12 June 2015
Received in revised form
7 September 2015
Accepted 10 September 2015
Available online 12 September 2015

Keywords:
Fiber optic biosensor
Tilted fiber Bragg grating
Surface plasmon resonance
Cytokeratin 7 biomarker
Small biomolecule detection

ABSTRACT

This study reports on the development of a surface plasmon resonance (SPR) optical fiber biosensor based on tilted fiber Bragg grating technology for direct detection of small biomarkers of interest for lung cancer diagnosis. Since SPR principle relies on the refractive index modifications to sensitively detect mass changes at the gold coated surface, we have proposed here a comparative study in relation to the target size. Two cytokeratin 7 (CK7) samples with a molecular weight ranging from 78 kDa to 2.6 kDa, respectively CK7 full protein and CK7 peptide, have been used for label-free monitoring. This work has first consisted in the elaboration and the characterization of a robust and reproducible bioreceptor, based on antibody/antigen cross-linking. Immobilized antibodies were then utilized as binding agents to investigate the sensitivity of the biosensor towards the two CK7 antigens. Results have highlighted a very good sensitivity of the biosensor response for both samples diluted in phosphate buffer with a higher limit of detection for the larger CK7 full protein. The most groundbreaking nature of this study relies on the detection of small biomolecule CK7 peptides in buffer and in the presence of complex media such as serum, achieving a limit of detection of 0.4 nM.

1. Introduction

Biomarkers are becoming increasingly important for therapeutic decision, in particular, in the cancer diagnosis. Protein biomarkers appear to be excellent indicators since they are correlated to the presence of pathogenic processes and diseases. Furthermore, their detection remains the most specific and sensitive technique at early stage of cancer. Consequently, protein biomarkers detection is a promising tool for the first-phase tumor detection.

Numerous technologies are employed to identify biomarkers, including the genetic and proteomic analysis (Kisluk et al., 2014), the immunohistochemistry (Pohl et al., 2014) and the quantitative polymerase chain reaction "Q-PCR" (Inoue et al., 2012). Most of the patient materials for biomarkers detections are post-operative tissue samples. Biopsies are applied when a cancer is suspected and consequently are realized often at an advanced stage of the disease. Furthermore, surgeon interventions are invasive and cannot be used repeatedly.

Investigations of protein biomarkers can also be realized on biological fluids, such as blood, pleural effusion or urines (Silva et al., 2012; Kisluk et al., 2014). Fluids not only have a great advantage due to their accessibility but can reflect the pathological process as well. Blood sample's analysis, for example, is of great interest since tumors are known to release biomarkers into the bloodstream, circulating tumor cells and fragments of tumor-cell DNA. However, liquid samples present a relative stability since proteins are not stable in non-physiological conditions. Furthermore, they are complex samples that need to go through different purification steps before analysis.

Generally, the current diagnostic tools are time-consuming and are therefore not applicable for intraoperative diagnosis. The search of an appropriate technology platform in the aim to improve biomarkers detection for early diagnosis has led to a new rapid method based on a label-free detection of biomarkers thanks to biosensors. These analytical devices are made up of two parts: a bioreceptor that detects an analyte and a transducer that transforms the signal resulting from the biological interaction into a quantifiable signal (Perumal and Hasdhim, 2014). Recently, several biosensors such as electrochemical (Silva et al., 2014), piezoelectric (Arif et al., 2015; Uludag and Tothill, 2010) and optical probes (Mukundan et al. 2009) have been employed to detect biomarkers

* Corresponding author at: Proteomics and Microbiology Laboratory, University of Mons, Mons, Belgium.
** Corresponding author.
E-mail addresses: clotilde.ribaut@umons.ac.be (C. Ribaut), christophe.caucheteur@umons.ac.be (C. Caucheteur).

http://dx.doi.org/10.1016/j.bios.2015.09.019
0956-5663/

which offer a direct and quantitative detection of synthetic analytes.

In the literature, many works have been published on biosensors using surface plasmon resonance (SPR) as transducing mechanism (Homola, 2008; Patching, 2014; Velasco-Garcia, 2009). In fact, SPR sensing has been demonstrated to be an exceedingly powerful and quantitative probe for the interactions of a variety of biomolecular interactions, including protein/ligand. An important advantage of SPR is its high sensitivity without any labeling of the interactants, coupled with an acquisition of data extremely rapid. SPR based biosensors have been used to detect a large variety of analytes including cells, DNA and various cancer biomarkers (Altintas and Tothill, 2013), which correspond most often to large biomolecules. However, it is interesting to note that most of the SPR biosensors have been used to detect full proteins and very few for targeting small biomolecules. To explain the reasons, their operating principle consists in the excitation of a surface plasmon at the interface between a metal film and a dielectric medium. SPR sensor relies thus on the monitoring of refractive index changes at the surface of the sensor and is consequently mass dependent. Most of the SPR biosensors work according to the Kretschmann Prism compatible with flat surface (Gopinath, 2010).

More recently, a new promising class of SPR biosensors has emerged, the optical fiber (OF) biosensor (Espinosa Bosch et al., 2007; Velasco-Garcia, 2009). Fiber-optic SPR sensors present the highest degree of miniaturization compared to all other SPR sensors (Homola, 2008). Different configurations allow to excite surface plasmons at the nanometric metal coated surface of the OF when light is brought into contact with the surrounding medium (Caucheteur et al., 2015a, 2015b). The most often encountered fiber-optic sensors based on SPR are made of unclad fibers covered with a nanometric layer of gold (Pollet et al., 2009; Shrivastav et al., 2015). In addition, the fiber grating technology such as long period grating (LPG), or tilted fiber Bragg grating (TFBG) (Albert et al., 2012; Caucheteur et al., 2013, 2015b) also allows to excite SPR. To summarize, upon contact with the grating photo-inscribed inside the optical fiber, light confined in the core is outcoupled and consequently enters in contact with the nanometric metal layer, coated in the central region of the grating, creating excitation of the plasmon resonance. Our interrogation system made of TFBG, illustrated in Fig. 1A, has several unique advantages (Albert et al., 2012). First, measurements are realized at the near infrared wavelengths between 1500 and 1600 nm with a spectral width of the resonance between 0.01 and 0.1 nm, yielding a high Q-factor with unprecedented figure of merit. A high Q-factor is important since it arises both the sensitivity and the limit of detection of the sensor. It also ensures a full compatibility with the telecommunication-grade equipment. Second, with near infrared TFBGs we are able to compensate temperature fluctuations thanks to the presence of the core mode resonance in their amplitude spectrum, the so-called Bragg mode, as depicted in Fig. 2A. Last but not least, TFBGs allow a straightforward injection of polarized light, which is essential for proper SPR generation, as it will be highlighted in the following.

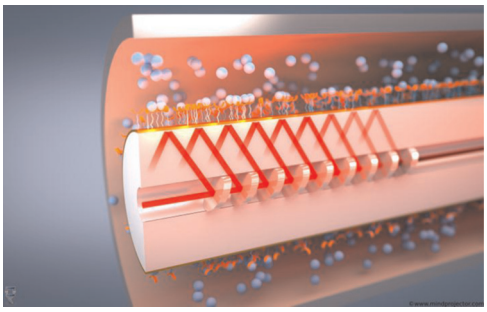

Fig. 1. Principle of the experimental set-up with a scheme of the optical fiber made of a cladding covered with antibodies immobilized on a nanometric layer of gold, and a core with tilted fiber Bragg grating inscribed in.

Gold-coated optical fiber biosensors, based on TFBG technology, have been already reported and the most crucial aim is attaining non-invasive and effective diagnosis (Albert et al., 2013; Guo et al., 2014; Shevchenko et al., 2011; Voisin et al., 2014; Malachovska et al., 2015). In fact, the micrometer size of the optical fiber biosensor offers new horizon, notably in biomedical applications such as introduction into a catheter, which can be of interest for early cancer diagnosis. Once again, optical fiber biosensors have been tested in the presence of large proteins. In view of the good sensitivity of this technology, we propose here to test with small biomolecules. To do so, we have focused our study on the lung cancer diagnosis.

A diverse range of tumor markers has been associated with lung cancer (Altintas and Tothill 2013; Flores-Fernandez et al., 2012). In this study, we focused on cytokeratins 7 (CK7). In fact cytokeratins are particularly useful tools for diagnosis in oncology (Sawant et al., 2008). In particular, CK7 profile of lung tumor has proved to be a useful aid in the differential diagnosis of carcinomas, since primary and metastatic tumors present different profiles. In fact, primary lung tumors express cytokeratin 7 (CK7+) while secondary tumors are deficient in CK7 (CK7−) (Campbell and Herrington, 2001). Moreover, it has been demonstrated that cytokeratin fragments can be released from malignant cells and consequently CK fragments can be located in blood circulation (Man et al., 2014) and are therefore easily accessible with an optical fiber properly modified.

In order to selectively detect cytokeratin 7 target with the SPR-TFBG, the gold coated optical fiber has to be functionalized. The development of a bioreceptor remains a crucial step, since stability and hardiness of bioreceptor immobilization onto optical fiber govern the biosensor performance. In fact, the recognition element immobilization rules specificity, selectivity, sensibility and reliability of the biosensor response. Among the several molecular edifices to detect cytokeratin proteins, our choice is focused on the commonly used antibody/antigen cross linking thanks to a first self-assembled monolayer (SAM) of thiols immobilized onto gold. SAMs are well-defined and described in the literature (Ulman, 1996; Love et al., 2005). SAMs are highly ordered and oriented and have the advantage to incorporate a wide range of groups both in alkyl chain and at the chain terminal, especially carboxylate function used to covalently immobilize antibodies thanks to a peptide bond. Furthermore, the biocompatible nature of SAMs makes them excellent structures for biomedical applications.

The cytokeratin 7 antigen detection by the bioreceptor is based on the specific chemical reaction with its corresponding antibody (AbCK7). The operating principle is based on the recognition of the cytokeratin 7 antigen epitope by the fragment antigen-binding (Fab), a region present on the cytokeratin 7 antibody. In addition to the full protein detection made of 469 amino acids, corresponding to a large biomolecule, we propose here to monitor a protein fragment called cytokeratin 7 peptide (CK7pep) made of only 23 amino acids in order to present a comparative study depending on the size of the target. In fact, the detection of small molecule antigens remains a challenge in the SPR immunosensing. Most of the SPR biosensors are based on large molecule detection since SPR response in presence of small mass suffers from various disadvantages not encountered with full protein, such as low signal-to-noise ratio. Nevertheless, new studies have proposed to offer small molecule SPR fiber-optic biosensors as proposed by Singh et al. with the detection of phenolic compounds (Singh et al., 2013). Our work features the first experimental evidence about reliable detection of small proteins with near-infrared plasmonic optical fiber biosensors. In addition to the small biomolecules