Letter

Development of surface plasmon resonance (SPR) biosensors for use in the diagnostics of malignant and infectious diseases

S Firdous 1,3,4, S Anwar 1,2 and R Rafya 1

1 National Institute of lasers and Optronics (NILOP), P.O. Nilore, 45650 Islamabad, Pakistan
2 National Center for Nanoscience and Technology (NCNST) University of Chinese Academy of Sciences (UCAS) 100190 Beijing, People’s Republic of China

E-mail: shamaraz@gmail.com and shamaraiz@nilop.edu.pk

Received 7 November 2017
Accepted for publication 5 March 2018
Published 26 April 2018

Abstract

Surface plasmon resonance (SPR) has become an important optical biosensing technology due to its real-time, label-free, and noninvasive nature. These techniques allow for rapid and ultra-sensitive detection of biological analytes, with applications in medical diagnostics, environmental monitoring, and agriculture. SPR is widely used in the detection of biomolecular interactions, and improvements are required for both sensitivity and in vivo uses for practical applications.

In this study, we developed an SPR biosensor to provide a highly sensitive and specific approach to early-stage detection of viral and malignant diseases, such as cancer tumors, for which biomarker detection is very important. A cancer cell line (HeLa cells) with biomarker Rodamine 6G was experimentally analyzed in vitro with our constructed SPR biosensor. It was observed that the biosensor can offer a potentially powerful solution for tumor screening with dominant angular shift. The angular shift for both regents is dominant with a time curve at a wavelength of 632.8 nm of a He–Ne laser. We have successfully captured and detected a biomarker in vitro for cancer diagnostics using the developed instrument.

Keywords: optical detection of diseases, surface plasmon resonance (SPR) biosensor, HeLa cell, tumor marker

(Some figures may appear in colour only in the online journal)
therapy response [5]. Rapid diagnosis is crucial to the effective treatment of any disease. Biological markers, or biomarkers, have been widely used to diagnose a variety of infectious and non-infectious diseases. The detection of biomarkers in patient samples can also provide valuable information.

An optical surface plasmon resonance (SPR) biosensor can detect the refractive index changes on the surface of sensor chips, label-free and in real-time. The main purpose of the development of SPR biosensors is to introduce an analytical instrument offering low cost, small size, quick and easy use, as well as a sensitivity and selectivity greater than the current instruments. This light-based SPR biosensor can be used for the early detection of malignant and viral diseases and health care monitoring, and enables more in-depth diagnosis, clinical analysis, veterinary and agricultural applications, industrial monitoring, and environmental pollution control.

**Material and method**

A HeLa cell line (derived from human cervical adenocarcinoma) was cultivated in minimum essential medium (MEM) containing 10% fetal bovine serum and 2 mM L-glutamine along with some nonessential amino acids. The cells were maintained at 37 °C in a moist environment as a sub-confluent monolayer in 25 cm² tissue culture flasks (Nunc, Wiesbaden, Germany) and were routinely sub-cultured twice or thrice weekly. The cell culture, with 70%–80% confluence wash, was harvested using 0.25% trypsin. The biomarker Rhodamine 6G is a tracer dye within water, having the chemical formula C_{28}H_{31}N_{2}O_{3}Cl and water solubility of 20 g l⁻¹ at 25 °C, and is used with HeLa cells in a flow channel [6–23].

SPR is an optical non-destructive method that can detect very small changes in the refractive index. It contains the
following components: a light source, prism, gold film, and detector, as shown in figure 1. We used the Kretschmann configuration of SPR, with a thin coating of gold film as a sensor chip over the base of the prism. A He–Ne laser of wavelength 632.8 nm and power 20 mW was used as the light source. When a light beam propagates in the prism and encounters the interface of the gold film and the solution, total internal reflection (TIR) takes place and an evanescent wave forms, provided that the incident angle is greater than the critical angle. Usually, the intensity of the reflected light does not change with the incident angle under the condition of TIR. However, at a specific angle larger than the critical angle, the evanescent wave excites the delocalized electrons or plasmons of the gold film and the intensity of the reflected light decreases sharply at this point. The incident angle at which the minimum reflectivity is observed is the SPR angle, as shown in figure 3 [24].

**Results**

SPR occurs when a photon of incident light hits a gold-coated surface. At a certain angle of incidence, a portion of the light energy couples, through the metal coating, with the electrons in the metal layer on the prism, which move due to excitation. The electron (plasmon) propagates parallel to the metal surface. The plasmon oscillation in turn generates an electric field between the metal surface and sample solution. Consequently, when there is a small change in the refractive index of the sensing medium, plasmon in the form of an evanescent wave cannot be formed. The evanescent wave arises due to total internally reflected light, when it meets the interface of the surface and a surrounding medium with a lower index of refraction. The evanescent wave decays exponentially with distance from the surface.
An angular dependence shift of the SPR from the HeLa cell monolayers with different cell coverages was observed. In the absence of cells, angular shift for total internal reflection is of 60.1°, which corresponds to the reflectivity from the glass and gold interface. In the presence of HeLa cells, the SPR dip appears at 62.15° and upon addition of the biomarker it is 64.5°, as shown in figures 2 and 3. The intensity angle is measured and plotted against time, as given in figure 4. At the beginning, a baseline is observed at 60.1°, where the minimum intensity angle is constant. At 62.15° a change occurs and the minimum intensity angle shifts for the cell line, and at 64.5° for both the cell line and biomarker. This is due to a change in the refractive index of the medium within the evanescent field. At the end, the minimum angle starts to shift back towards the baseline.

Initially we confirmed the SPR angle by repeatedly performing the experiment that produced a result of 60.1°. The HeLa cell line is used as a biological recognition element into the flow cell. From the angular shift observed at 62.15°, and by then injecting Rhodamine 6G as a biomarker, we observed the shift in SPR angle to 64.5°, due to an increase in the refractive index of the sensor chip surface. This is due to the fact that the SPR is very sensitive, and even small changes in the measured surface cause a shift in the SPR curve.

Haes et al presented an SPR biosensor for the determination of biomarkers of Alzheimer’s disease. This is a progressive mental disorder disease encountered worldwide, and as no specific cure is presently available, early diagnosis is a crucial matter for existing drug treatments. Based on a sandwich assay format, the proposed biosensor showed quantitative binding information for both antigen and secondary antibody detection, allowing the determination of the higher concentrations found in patients with disease, in comparison to control patients [25].

Park et al discuss a SPR biosensor using a multi spot gold-capped silica nanoparticle array chip for the detection of avian influenza infectious viral disease. A metal binding polypeptide (GBP)-fusion protein was bound onto the gold substrates by means of specific interactions, allowing the immobilization of proteins in bioactive forms onto the gold surface, and suitable for immunosensor applications [26]. A highly sensitive SPR immunosensor was proposed for the detection of HIV virus, based on the realization of a nanopattern of circular gold nanodots which was electrodeposited onto a glass substrate coated with indium tin oxide [27]. This approach could be useful for the development of biochips for the analysis of the detection of several viruses.

Conclusions

The locally constructed optical biosensor will be used for the early-stage detection of diseases in the healthcare, biomedical, and biopharmaceutical sectors. It will be a new analytical tool, of reduced size, and facilitating the large-scale sensitive screening of a wide range of samples for many different parameters. Optical biosensors have been successfully tested in many fields, such as medicine, pharmacy, food safety, environment, biotechnology, defence, and security. In our opinion, the synergic effect in the field of optics, nanotechnologies, microfluidics, biotechnologies, and surface chemistry will result in the development of improved SPR biochips which will be able to be used as an alternative to the traditional methods commonly employed for in vitro diagnostics.

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