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Review

Optical biosensing for label-free cellular studies

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ABSTRACT

Optical biosensors such as waveguides and surface-plasmon resonance (SPR) sensors have found numerous applications in biomolecular sciences. We provide an overview of these technologies in relation to the specific requirements of label-free and high-throughput cellular studies. We dedicate specific emphasis to SPR-based biosensors and recent developments particularly suitable for cellular studies, such as long-range SPR. We discuss the advantages and the disadvantages of the most successful optical-sensing technologies, and potential approaches in the next generation of optical technologies.

Contents

1. Introduction

Due to their intrinsic label-free nature and ability to provide sensitive and specific detection of biomacromolecules in real-time, solid-state optical biosensors have become essential in medical diagnostics, drug screening, food quality and safety, environmental protection, biotechnology and biohazard security [1,2]. Amongst the optical biosensor family, the most commonly utilized are surface plasmon resonance (SPR) and waveguide biosensors. Several thousand research articles have been published investigating genetic materials, enzymes, proteins and chemical compounds using optical biosensors. Since its launch in 1990, the Biacore SPR platform has become a mainstream bio-analytical technology,

Abbreviations: cLRSPR, Coupled long-range surface-plasmon resonance; EGFR, Epidermal growth factor receptor; GPCR, G protein coupled receptor; IC_{50}, Inhibitory concentration at 50%; IRSPR, Infrared surface-plasmon resonance; LRSPR, Long-range surface-plasmon resonance; LSPR, Localized surface-plasmon resonance; NGWSPR, Near guided wave surface-plasmon resonance; QCM, Quartz-crystal microbalance; RI, Refractive index; SPR, Surface-plasmon resonance; SPRi, Surface-plasmon resonance imaging; SPRM, Surface-plasmon resonance microscopy; SPR-PI, Surface-plasmon resonance phase imaging; TE_0, 0th order transverse electric mode; TM, Transverse magnetic polarization; TM_0, 0th order transverse magnetic mode; TM_1, 1st order transverse magnetic mode.

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present in most leading research centers, global pharmaceutical companies, and many biotechnology companies. The optical biosensor field is a very dynamic one, and many new technologies are likely to be commercialized in the next few years, further broadening the applications of optical biosensing in the life sciences.

One significant disadvantage of endpoint molecular methods, such as ELISA and PCR assays, is their static nature, which does not provide kinetic information [3]. However, optical biosensors easily provide real-time measurements and are intrinsically label free, eliminating the need to modify the primary or secondary target with a probe. That makes the procedure and the readout more straightforward and less prone to possible artifacts arising from labeling [4,5]. They also have great potential for high-throughput screening, automation, and integration into microfluidic lab-on-chip concepts [6]. In bioanalytical studies, optical biosensors have therefore gradually complemented, or even replaced in some instances, the established molecular methods [3].

Following the success of optical sensing in studying molecular interactions, the inherently label-free nature of optical-sensing technologies combined with their high sensitivity and ability to provide real-time information, more recently, inspired their application to the study of microorganisms, such as mammalian cells and bacteria. One can broadly distinguish two different types of studies in this field. The first is the detection of target microorganisms from a complex sample through their specific binding to the sensor surface, which has found some practical applications (e.g., in the food industry) [7]. Optical-biosensing approaches have also been successfully used in cell-based assays, where the response to an external stimulus (e.g., drug and protein therapeutics) of cells seeded onto the sensor surface is monitored in real time, thereby providing a powerful screening technology (e.g., for cellular responses in drug discovery). Commercial systems for cell-based assays relying on waveguide technology have been developed and commercialized {e.g., the Epic system from Corning [8] or the Bind technology from SRU Biosystems [9]}. Although such approaches can provide ultra-sensitive, label-free, real-time measurements about complex intracellular events, their main shortcoming is that only limited information can be obtained about the biomolecular nature of the observed responses. Consequently, additional endpoint studies are required to elucidate, if necessary, the molecular pathways at play in the observed response. While this review does not discuss non-optical, label-free, biosensing technologies used for cellular studies, the interested reader is referred to key studies using quartz-crystal microbalance (QCM) [10–13] and impedance-based biosensing [3,14,15].

This article provides an overview of the exciting potential of optical-biosensing technologies in the study of microorganisms, such as mammalian cells and bacteria. It aims to provide an introduction to optical biosensors, such as waveguide and SPR, and to describe the structure of these sensors and their response to the presence of large biological entities. In the final section, we discuss improvements to the current approaches and novel technologies with a focus on technologies enabling enhanced penetration into the sensing medium.

2. Characteristic features of optical biosensors for living microorganisms

Optical sensors are sensitive to changes in the refractive index (RI) – the optical density – in the vicinity of their active surfaces. For all optical biosensors, one can define the sensitivity as a function of changes in the adjacent bulk solution, of the thickness of an adlayer on the surface and of the change in the RI of this adlayer, as per Equation (1):

$$dN_{eff} = (\partial N_{eff}/\partial n_s).dn_s + (\partial N_{eff}/\partial n_a).dn_a + (\partial N_{eff}/\partial d_a).dd_a \quad (1)$$

where N_{eff} is the effective RI of the optical structure, n_s the RI of the cover medium, n_a the RI of the adlayer and d_a its thickness.

The physical sensitivity of a device is defined as the variation of the effective RI of a structure to one of the three aforementioned parameters. Waveguides and SPR sensors are among the most sensitive sensing technologies. This means that a small change in one of the three variables triggers a significant variation of N_{eff}, which is eventually converted into the readout signal.

The ability of optical biosensors to measure with high sensitivity and in a label-free fashion the local change of RI can therefore readily be used to detect the binding of microorganisms onto the sensor surfaces. The microorganisms are sensed as a mass increase due to the local change in the RI, as cells have a higher RI than aqueous fluids, such as serum. Importantly, however, cells and bacteria have dimensions greater than the typical penetration depth (100–200 nm) of standard optical-sensing technologies into aqueous media, and, consequently, only a small portion of the entire volume of cells close to the sensor surface is sensed (Fig. 1e). As we discuss later in this review, optical biosensors have, however, been applied successfully (e.g., in the food industry for the rapid detection of potentially harmful bacteria). But, beyond these more traditional sensing applications, some unique features of optical biosensors have, when applied to living microorganisms, been exploited to design advanced cell-based assays.

At the scale of evanescent-wave penetration, cells and bacteria are intrinsically optically inhomogeneous entities, since the various cellular components have different optical properties. For example, the cell-membrane RI is of the order of 1.48, while the cytoplasm has an RI of 1.38 [16]. As to other key cellular components, proteins have a range of RI that falls between the liquid-medium RI and the dry-protein RI of 1.53, depending on their density and hydration [17]. Most studies have considered the "average" RI of a whole cell to be 1.35–1.38 [16,18] (e.g., 1.36 for Chinese human ovary cells [19]). It is noteworthy that these differences in RI values are easily detected by optical biosensors, as their sensitivities enable accuracy to, at least, the fourth decimal place.

Another important aspect of optical biosensors is that their sensitivity greatly depends on the distance to the sensor surface, since the electromagnetic fields responsible for the sensing typically decay exponentially with distance from the surface. For biological entities larger than the evanescent field, this translates into lesser sensitivity the further away from the surface. Fig. 1 illustrates the principle of optical biosensing in cellular study schemes.

Importantly, these two aspects of optical biosensing – the sensitivity to the various RIs of cellular components and the distance-dependent sensitivity – enable monitoring of complex biomolecular rearrangements within the cell that can ultimately be linked to multifaceted intracellular pathway signaling. Optical biosensors are therefore powerful tools for the detection of such intracellular events. Optical biosensors are also efficient at detecting morphological changes and can be utilized for monitoring cell adhesion, spreading, cytoskeleton remodeling, and migration.

Furthermore, since morphological changes can be related to cell viability, biosensors are well suited to cytotoxicity assays, drug discovery, and anti-viral strategies.

3. A brief overview of waveguide and SPR biosensors

Waveguides are multi-layered structures that confine one or more electromagnetic waves (e.g., light from a laser) within an inner guiding layer, via the control of interfacial RI differences of