REVISTA DE INVESTIGACIÓN CLÍNICA

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PERMANYER

Rev Invest Clin. 2019;71:85-90 PERSPECTIVES

Clinical and Biomedical Applications of Surface Plasmon Resonance Systems

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ABSTRACT

Surface plasmon resonance (SPR)-based biosensors offer superior analytical features such as simplicity, sensitivity, and specificity when compared to conventional methods in clinical analyses. In addition, they deliver real-time monitoring of label-free analytes with high-throughput approaches requiring little sample pretreatment that allows the analysis of virtually every clinical sample type to determine the amount and/or activity of any molecule of interest. Accordingly, SPR emerges as a novel, efficient, powerful, and relatively low-cost alternative tool for routine clinical analysis, opening also new horizons for developments in personalized medicine applied to diagnostics or therapeutics' monitoring. (REV INVEST CLIN. 2019;71:85-90)

Key words: Surface plasmon resonance. Biomarker. Clinical diagnostics.

INTRODUCTION

Biosensors are analytical devices consisting of a biological sensing element connected to a physicochemical transducer. These elements generate digital electronic signals proportional to the interaction of biomolecules, thus allowing "label-free" detection of analytes. Due to the specificity of biomolecular interactions, a biosensor can be used to analyze complex substrates including samples such as blood, serum, plasma, urine, milk, and culture media often with minimum preparative treatment.

Different types of biosensors have been developed including amperometric, potentiometric, piezoelectric, calorimetric, and optical biosensors. These have been applied mostly in food and water analysis and pharmaceutical processes due to their demands for sensitivity, specificity, speed, and accuracy of analyte measurements. Among them, optical biosensors correlating changes in concentration, mass, or number of molecules to direct changes in characteristics of light have preferentially evolved in these past years. Optical detection using surface plasmon resonance (SPR) biosensors has been increasingly popular due to its

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Received for publication: 28-08-2018
Approved for publication: 25-09-2018
DOI: 10.24875/RIC.18002754

speed of detection, high specificity, high sensitivity, and possibility of real-time analysis[1].

SPR-BASED BIOSENSORS

SPR appeared as a revolutionary technology > 20 years ago, when their first commercial instrumentation, the Biacore, was launched on the market by the Swedish company Pharmacia Biosensors AB[2]. Since then, many researchers adopted the SPR in various analytical fields such as the food industry, pharmaceutical approaches, doping analysis, proteomics and genomics, and environmental monitoring. Beyond that, clinical and biomedical analyses have also been explored with promising results[1,3].

In a typical SPR experiment, one interacting molecule, referred as the "ligand," is bound to the biosensor surface while the other, called "analyte," is delivered to the surface in a continuous flow through a complex microfluidic system. The biosensor consists of a glass piece coated with a layer of gold, creating a platform for a range of specialized surfaces designed to optimize the binding of a variety of molecules including inorganic compounds, proteins, lipids, nucleic acids, carbohydrates, and even whole cells. The gold layer in the biosensor allows the generation of the SPR events that essentially detect changes in mass in the aqueous layer close to the biosensor surface by measuring changes in the refractive index of an incident polarized light beam (Fig. 1). The data obtained provide real-time quantitative information about binding specificity, active concentration of a molecule in a sample, kinetics, and affinity of binding models, among others. The complex theory behind SPR has been extensively detailed before[4]; thus, it will not be further discussed here.

SPR APPROACHES FOR BIOMEDICAL AND CLINICAL APPLICATIONS

SPR analyses possess a great potential for clinical and biomedical applications due to their inherent advantages when dealing with biomolecules. Optical SPR-based studies can be carried out with colored or even opaque samples, and there is no need to label molecules of interest with fluorescent or radioactive tags, thus avoiding the possibility that labels may compromise molecular activity or association features. In this way, analytes derived from clinical samples, human cells, or tissues can be studied in their native state to provide results that reflect more accurately their activity *in vivo*[3,5].

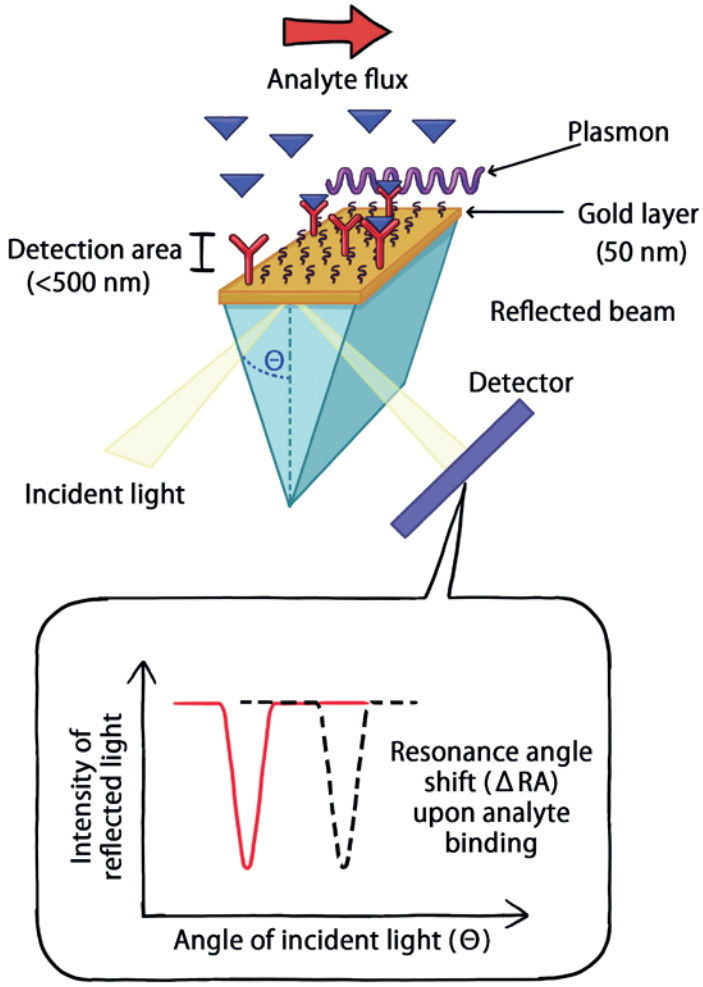
In recent years, there has been a significant increase in research articles reporting the development of SPR techniques for biomolecular analysis; accordingly, several studies report the detection of different peptides, proteins (including antibodies), hormones, microRNA, and DNA. Consequently, multiple SPR-related reports emerged showing the analysis of clinical samples from patients suffering specific diseases, which is usually the ultimate objective of biomedical researchers looking for new diagnostic tools.

SPR approaches have been applied to clinical tests for monitoring antibodies, proteins, enzymes, drugs, small molecules, peptides, nucleic acids, and even bacterial components or viruses. Amazingly, while the molecules detected were usually at concentrations of nanomolar scales or higher, several studies reported picomolar detection or even lower concentrations. This technique has been applied in a number of biofluids including plasma, serum, whole blood, urine, stools, saliva, cervicovaginal secretion, endometrial tissue, synovial, cerebrospinal, amniotic, and ascites fluids[1,3,6-9]. The analytes detected by SPR in these samples represent an everyday growing list of molecules with great potential for clinical-related applications, as exemplified in the next sections and detailed in Table 1.

Protein Biomarkers

A protein biomarker is used in clinical diagnosis to monitor the status of several associated diseases, displaying a potential utility for targeted therapy and to evaluate the therapeutic responses. The implementation of proteomic approaches together with the growing number of clinical biomarkers of proteic nature is accelerating the development of reliable methods for their detection in complex clinical samples such as SPR techniques. Examples of these clinically relevant protein biomarkers include molecules related with specific diseases[1,3,6-9], as explained below.

Figure 1. Fundamentals of surface plasmon resonance (SPR) sensor. SPR is a physical phenomenon that occurs when polarized light strikes an electrically conducting surface (made of gold in most cases) at the interface between two media, a high-refractive-index glass or crystal prism and a low-refractive-index buffer solution. This generates intermittent waves of electrons (charge density waves), also known as plasmons, that reduce the intensity of reflected light at a specific angle known as the resonance angle (RA), in proportion to the mass deposited on the conducting surface. These arrays, referred to as SPR sensors, are used to detect a refractive index change of the polarized light beam within a detection area (< 500 nm) as a change of the RA, when the interactions between ligand molecules (attached to the sensor) and the analytes (flowing in solution) occur.



Cancer Biomarkers

An important aspect of all types of cancer management should include the monitoring of protein biomarkers related with these diseases, preferentially in easily collected physiological fluids over surgically obtained biopsies; examples of these proteins are the prostate-specific antigen, podoplanin, lipocalin 2, galectin 1, and CD166 (ALCAM), among others. Besides providing practical information to guide clinician's decisions, cancer biomarkers are also linked to specific alterations in molecular pathways controlling cancer pathogenesis, thus evidencing their potential for deciding about therapeutic strategies[8,9].

Cardiac Disease Biomarkers

Cardiac biomarkers such as C-reactive protein, brain natriuretic peptide, myoglobin, and cardiac troponin I are proteins released into the bloodstream on damage of the heart or associated tissues. These biomarkers help to diagnose acute coronary syndrome

Table 1. Clinically relevant analytes determined by SPR analyses.

Disease or condition	Analytes measured by SPR	Sample
Antibodies		
Antiphospholipid syndrome	Anti-cardiolipin, anti-β2 glycoprotein I	Serum
Chagas disease	Anti-*Trypanosoma cruzi*	Serum
Dengue	Anti-dengue virus IgM antibodies	Serum
Diabetes	Insulin and proinsulin autoantibodies	Serum
Epstein–Barr virus infection	Anti-VCA, anti-EBNA, and anti-EA, all viral antigens	Serum
Hepatitis A and B	Anti-hepatitis A or B antigens	Serum
Leukemia	Anti-asparaginase, κ and λ immunoglobulin light chain	Serum
Lyme disease	Anti-*Borrelia* spp.	Serum
Neonatal thrombocytopenia	Anti-HPA-1a alloantibodies	Serum
Peanut allergies	IgE	Serum
Red cell aplasia	Anti-erythropoiesis-stimulating agent IgG4 antibodies	Serum
Rheumatoid arthritis	Anti-glucose 6-phosphate isomerase, anti-citrullinated protein antibodies	Synovial fluid and serum
Syphilis	Anti-*Treponema pallidum*	Serum
Systemic lupus erythematosus	Anti-dsDNA autoantibodies	Serum
Typhoid fever	Anti-*Salmonella enterica* serotype typhi	Serum
Other proteins		
Alzheimer's disease	Tau protein	Serum
Bladder cancer	Podoplanin	Serum, urine
Cancer	Galectin-1	Serum
Cardiopulmonary bypass surgery	IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ	Serum
Diabetes	Insulin	Serum
Head and neck squamous cell carcinoma	p53, p38αMAP kinase	Serum
Heart disease	C-reactive protein, BNP, MG, and cTnI	Serum
Hematopoiesis	CXCL12	Urine
Hepatocellular carcinoma	Lipocalin-2	Serum
Lung cancer	Rac	Serum
Osteoarthritis	TNF-α, MMP-3	Synovial fluid
Pancreatic cancer	ALCAM	Serum
Preeclampsia	Albumin	Urine
Prostate cancer	PSA	Serum
Rheumatoid arthritis	Cathepsin G	Endometrial tissue
Tuberculosis	CFP-10	Urine
Other molecules		
Alzheimer's disease	Amyloid-β	Cerebrospinal fluid
Cancer	Exosomes	Ascites fluid
Cancer treatment	Methotrexate	Serum
Celiac disease	Gluten peptides	Urine
Enterohemorrhagic *Escherichia coli* infection	PCR products of *Escherichia coli* O157:H7	Stool
Fertility monitoring	Estriol 3-sulfate 16α-glucuronide	Serum
Pancreatic cancer	miR-21 and miR-10b	Plasma
Sport doping	Human growth hormone	Serum
β-thalassemia	Genomic DNA	Blood

Molecules used in clinical approaches, detected in human samples by SPR methods. The table was adapted from Mariani and Minunni[3].
PSA: prostate-specific antigen, SPR: surface plasmon resonance, IL: interleukin, Ig: immunoglobulin, TNF: tumor necrosis factor, IFN: interferon, BNP: brain natriuretic peptide, MG: myoglobin, cTnI: cardiac troponin I, PCR: polymerase chain reaction.

and cardiac tissue ischemia, both conditions associated with insufficient blood flow. Cardiac biomarker proteins can also be used to estimate an individual's risk to develop these conditions or to help monitor and deal with a patient suspected to suffer from these conditions[3].

Antibodies

Although SPR has been extensively applied for the characterization of monoclonal antibodies, both used in therapeutics or biomedical research, its utility in diagnosis by measuring antibody levels in circulation is a neglected application. However, there are reports of SPR playing a crucial role mainly in the analysis of autoantibodies in human serum for real-time monitoring of autoimmune disorders including for example anti-dsDNA autoantibodies in lupus erythematosus and anti-citrullinated protein antibodies in rheumatoid arthritis[3].

Another field where antibody detection by SPR sensors could be important is the biopharmaceutical industry. Here, the immunogenicity of biological drugs is always a concern since they are primarily proteins (monoclonal antibodies, cytokines, growth factors, hormones, enzymes, or fusion proteins) and peptides that, when administered, often could induce a drug-specific immune response characterized by the presence of anti-drug antibodies (ADAs)[10].

ADAs have been detected in clinical studies resulting in significant alterations in toxicology, pharmacokinetics, and efficacy of biotechnology-derived pharmaceuticals. Consequently, regulatory authorities are now requesting immunogenicity studies that include the detection of ADAs, before the approval of any biological drug[10]. On this scenario, the use of SPR devices for ADAs detection represents a valuable approach due to the intrinsic and previously discussed advantages of this technology.

Hormones Measurement

The analysis of both lipidic and small peptide hormones is an important area in clinical diagnostics and, currently, in anti-doping regulations. Endocrine diseases, where these mediators are typically involved, usually require measuring hormone levels by direct or indirect methods. Accordingly, SPR has demonstrated the ability to provide sensitive solutions in this clinical area by determining, for example, estriol metabolites and human growth hormone, among other hormones[3,7].

Nucleic Acid Analyses

SPR assays related with nucleic acids could be subdivided into two main classes: analyses for the detection of chromosome abnormalities including point mutations and single-nucleotide polymorphisms such as those occurring in β-thalassemia, and assays for the quantification of genetic material as biomarkers such as microRNAs[3].

Pathogens Detection

Beyond the identification of pathogens through specific DNA sequences targeting, other approaches based on the detection of a number of cell pathogen components have been reported. Particularly, several bacterial and parasite components, including *Salmonella* spp. and *Schistosoma mansoni* antigens, or even whole of H1N1 influenza virus particles, have been subject to SPR analyses[6].

Whole-cell SPR Analyses

The detection depth of conventional SPR sensors reaches no > 500 nm beyond the gold layer; therefore, they are useful for the detection of changes near the plasma membrane of live cells with a high sensitivity. In this way, different SPR techniques have been developed to perform real-time evaluation of exogenous stimuli-induced responses in living cells. These approaches might reflect the reorganization of proteins as a consequence of intracellular signal transduction processes.

Examples of whole-cell analyses by SPR devices include the detection of real-time adhesion and morphological changes in living cells due to the action of toxins or enzymes[5]. A similar approach is the SPR measurement of the apoptosis rate of cancer cells in response to different oncological drugs, which can be applied in a clinical setting to evaluate the individual therapeutic potential of different treatments including pharmacodynamic interactions[5]. In addition, SPR sensors could reveal real-time alterations in intracellular signaling pathways of abnormal cells such as

cancer cells, making them able to detect malignant tumors[5].

Finally, the recently developed SPR imaging systems and the long-range SPR sensors for living cells may allow the visualization and deep through analyses of single cell reactions, potentially expanding the application of SPR whole-cell sensing for clinical diagnosis in the near future.

CONCLUSIONS

To be implemented as a routine analytical method, SPR sensing will need to replace existing technologies. In this way, several clinical results obtained with SPR devices have been compared to those from ELISA assays, chemiluminescence approaches, polymerase chain reaction, or liquid chromatography–mass spectrometry, generally demonstrating similar quality[8].

Since ELISA employs the same experimental design as SPR sensing, regarding the detection of analytes using direct, indirect, or sandwich assays, it is frequently the method against which SPR systems are compared. The studies show that ELISA and SPR deliver the same dynamic range of sensitivity, although the advantages of SPR reside in the label-free and rapid detection of analytes plus the possibility of reusing the same sensor many times, which could decrease the cost of serial measurements. The SPR method is also preferred for the detection of molecules with low binding affinities, which can otherwise be washed away in ELISA assays.

Although the detection of antibody and general protein markers dominates the current applications of SPR approaches that undoubtedly support medical practice, the increasingly recognized usefulness of genetic testing, microRNA detection, and special molecules' analyses in clinical practice could represent an interesting niche for SPR applications.

In the upcoming years, novel integrated, simpler, and even portable SPR systems, capable of detecting biomolecules with high sensitivity ideally in undiluted biofluids, will be surely available to clinicians for daily use, allowing for a more accurate decision-making in the management of a given patient. For now, the promising results of this technology for the analysis of human samples in detecting biomarkers and helping with diagnostic or prognostic approaches in several diseases position SPR techniques as a powerful tool in clinical management with a great potential in precision medicine.

ACKNOWLEDGMENTS

This work was supported by grants 240314 from CONACyT as well as IA204316 and IA202318 from UNAM-DGAPA-PAPIIT program. The authors thank Ari Kleinberg-Bild (RAI, UNAM) for the art work of Figure 1.

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