Surface plasmon resonance: a label-free tool for cellular analysis

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Abstract:

Surface plasmon resonance (SPR) is a popular technique that allows for sensitive, specific, label-free and real-time assessment of biomolecular interactions. SPR is a nondestructive, modular and flexible tool for various applications in biomedical sciences ranging from cell sorting, cell surface characterization and drug discovery. In this review, we will discuss more specifically how SPR is used to monitor the dynamics of various types of cellular binding events and morphological adherence changes in response to external stimuli.

Keywords: bacterial adherence properties | biomolecular interactions | cell sorting | cellular detection | cellular morphology alterations | cellular secretions | long-range surface plasmon resonance | nanoscale cellular dynamics | optical biosensor | single-cell analysis

Article:

As the field of nanotechnology continues to develop, sophisticated and sensitive tools are needed to evaluate single-cell activity as subtle cellular dynamics could be masked from measurements that only account for an average response of a heterogeneous cell population [1-3]. For example, in mixed cellular populations, the single-cell response can be concealed by the bulk response, thus deterring distinct and accurate measurements of cellular and molecular binding activity dynamics [4]. The ability to assess single-cell and molecular dynamics has helped in many areas of biological research; for instance, in gene expression mapping [5], cancer [6] and stem cell research [7]. Recently, researchers are investigating the potential of label-free and real-time monitoring systems such as surface plasmon resonance (SPR), quartz crystal microbalance and real-time impedance [8-14] to study single-cell activity under controlled conditions.

Introducing SPR as a sensitive platform to the areas of cell-surface interactions, cell-antibody interactions and intracellular responses due to extracellular stimulation has offered a promising potential for research and clinical diagnosis purposes [15-18]. In this review, we will highlight the progress of SPR in biomedical applications focusing on immunological sensing, monitoring biofilm, morphology and secretory responses at the cellular levels.

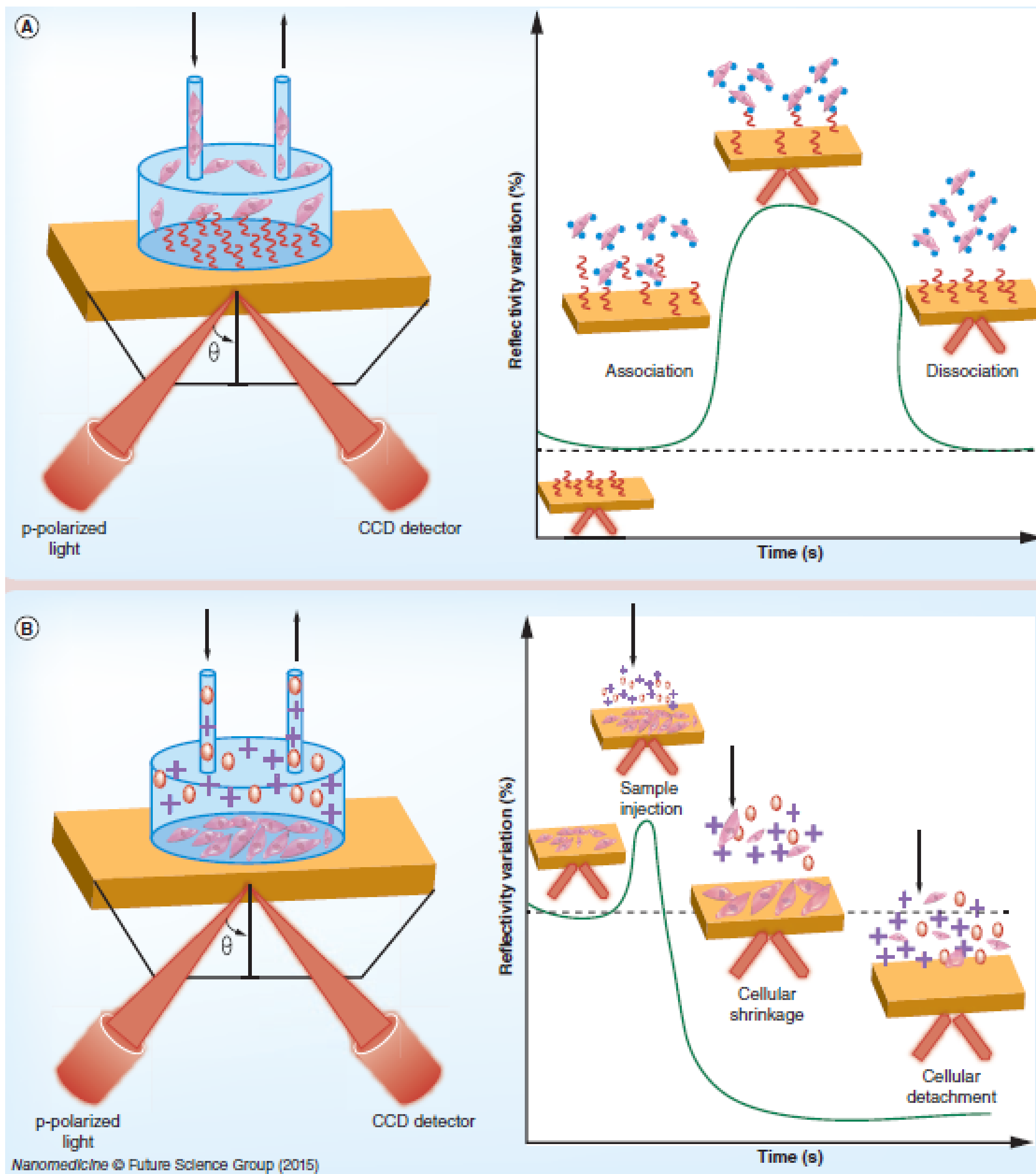

Figure 1. A schematic representation of the two possible modes for cellular analysis using surface plasmon resonance imaging. **(A)**Mode 1: (left) schematic of the surface plasmon resonance platform for selective detection of cells by immobilized capture ligands. (Right) Surface plasmon resonance sensorgram of the association and dissociation of the cell-ligand interactions. **(B)** Mode 2: (left) schematic of the surface plasmon resonance platform for monitoring cellular response upon stimulation. (Right) Surface plasmon resonance sensorgram of the corresponding signal changes due to morphological changes in the on-chip adherent cells.

SPR principle

SPR is a technique that enables the monitoring of biomolecular interactions in real time in a label-free manner. In general, SPR biosensors [19] consist of three major components: an optical system composed of a diode laser that shines light onto a gold-coated (50-nm thickness) prism with a high refractive index (RI; 1.517) and a detector that records the changes in reflectance.

Excitation of free electrons propagating on the metal surface occurs under attenuated total reflection conditions after a monochromatic p-polarized light interacts with the surface of the sensor chip at a specific incident angle. This in turn generates a dip in the reflectivity curve as a result of resonance energy transfer that occurs between evanescent wave and surface plasmons [20]. The resonance or SPR angle is defined as the angle at which the greatest loss of the reflected light intensity occurs. This SPR angle is extremely sensitive to any change or perturbation in the RI of the medium adjacent to the metal surface (<300 nm), and such changes can be monitored by the shift of the SPR angle. In turn, kinetic data are attained from changes in SPR angle, when the system goes out of resonance as a result of a biomolecule binding to the surface [21-24].

To respond to the need for an array format detection system, SPR imaging (SPRi) was developed [22,25] about a decade ago. In this case, the reflected light is intercepted by a high-resolution charged coupled device camera [22] generating a real-time digital image of the sensor surface, which translates a binding event to a change in contrast. This is accompanied with a kinetic sensorgram providing the percent change of reflectivity over time.

Extending the applications of SPR-based technologies toward obtaining information regarding cellular activity, such as structural and morphological alterations in the cells, emerged mainly by two strategies. The first one is performed by using the cells of interest as the injection sample within the device (Figure 1A) and the second one by immobilizing the cells on the surface of the biochip (Figure 2B). Several examples of how SPRi has been used for detecting cellular interactions are described.

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Figure 2. Surface plasmon resonance response of G-type biochip functionalized with 20 ug/ml epithelial cell adhesion molecule antibodies after the injection of MCF-7, SKBR3 and HS578T cells.

Application of SPR in the analysis of cell receptor-ligand interactions

The requirement to assess pathogens in food, medicine and environment has instigated the development of SPR cell-based biosensors that can provide mechanistic and kinetic insight [27-33]. For example, Watts *et al.* were one of the first groups to apply the SPR platform for the detection of whole living cells. They utilized an aminosilane and immunoglobulin G-functionalized surface to detect *S. aureus* (cowan-1) cells, which expresses protein A [34]. In this work, they highlighted that in order to achieve good sensitivity with their approach, the selection of immobilization method is of great importance as it influences the SPR-binding efficiency. More recent research efforts have also employed specific antibodies as the capturing ligands to selectively bind certain cells from blood samples. Suraniti *et al.* [35] were able to selectively detect B and T-lymphocyte cells using an antibody-coated chip binding directly to cell membrane glycoproteins. The targeted antimouse CD19 and CD3 antibodies were modified with pyrrole (chemical linkers) and immobilized on the biochip through electropolymerization. These antibodies target the CD19 and CD3 glycoproteins on the membrane of B-lymphocytes and T-lymphocytes, respectively. The authors noted that SPRi can only detect cells binding to the surface of the biochip; however, free-flowing cells are not detected. In turn, the SPRi platform is more suited for cell-surface adhesion kinetic analyses. Pyrrole-modified antibodies were also

later used by Milgram *et al.* [36] to test the selectivity of their platform for live cell capture purposes.

Another example of assessing surface antigen-antibody interactions was to monitor binding events of different cell lines [26]. For instance, Stojanovi *et al.* focused on the interaction of different cell lines that express EpCAM at varied densities on their surfaces with EpCAM-specific antibodies. MCF-7 breast cancer cells express EpCAM with a comparable density to SKBR3 cells; however, at a much higher density than the negative control (HS578T). Regions of interest (spots) on the sensor surface were immobilized with EpCAM antibodies and bovine serum albumin serving as a negative control. Different cell lines MCF7, SKBR3 and HS578T were injected over identical chips followed by a sedimentation phase to allow time for binding after the flow is restarted. The strongest SPR signal response was for MCF-7 followed by SKBR3 and HS578T cells exposed to the sensor surface that has been spotted with EpCAM antibodies. However, in the absence of EpCAM, the same trend was observed but the signal response was marginally lower (Figure 2). Interestingly, after the flow is restarted only MCF7 and SKBR3 signal increased, whereas HS578T experienced a minimal response. These results are consistent and attributed to the fact that MCF7 has the highest expression of EpCAM followed by SKBR3 and HS578T, respectively. This work introduces another novel application of SPRi in quantifying the density of surface antigens expressed by different cell lines [26]. A series of studies were performed in this area on different types of cell lines such as murine macrophages [37], or ABO blood-typing using different classes of antibodies [38]. These studies demonstrate the utility of SPR in conjunction with immune markers for the sensitive detection of cell populations that are present with relatively low concentration in whole blood.

An alternative hybrid approach to selectively capture live cells was introduced using a molecular assembly that incorporates DNA as the chemical linker between the surface of the chip and the capturing antibodies [36]. In this unique approach, the specific antibodies remained as the capturing ligand; however, DNA microarrays were combined in the surface molecular assembly. The pyrrole-NHS-modified DNA probes (pDNA) were grafted to the surface of the biochip using an electropolymerization process. Following this step, the antibodies of interest were conjugated to the target DNA (tDNA) and hybridized with the pDNA on the surface through an intermediate DNA (iDNA). The purpose was to study the capture of specific immune cells and their controlled release through the injection of specific cleavage enzymes to the on-chip construction [36]. The controlled release was further exploited for cell sorting applications [39]; in which, the construction of the DNA-protein complex was monitored by the SPRi and then applied to capture live primary cells and ultimately allow their release (Figure 3). In comparison to traditional methods like fluorescent-assisted cell sorting (FACS) [40] that allows you to separate and identify different types of cells, SPR does not offer the high-throughput FACS provide. However, SPR allows you to identify cell type in a label-free manner, whereas FACS requires labeling surface proteins prior to analysis. In addition, the SPR-based construct by Bombera *et al.* discussed above offers a promising potential for developing a new cell sorting device at the individual cell level and a simple way to monitor both molecular and cellular interactions simultaneously using the same device [39].

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Figure 3. Surface plasmon resonance imaging response of B- and T-lymphocyte attachment and enzyme-cleavage release (Zip1: *Eco*RI, Zip2: *Pvu* II, Zip3: *Nco* I).

Recently, several approaches aiming at detecting specific cells used protein peptides as the capture ligands [25,27]. This strategy has gained growing interest in developing drug discovery applications tailored for cell membrane receptors. A great example was presented by Mizuguchi *et al* . to understand the inhibitory function of certain drugs on pathological cells by evaluating the binding affinity of ligands to membrane receptors using SPR [41]. Epidermal carcinoma cells (A431), which highly express the EGF receptors, were used as the injected analyte directed to specifically bind the EGF immobilized on the surface of the biochip. The EGF peptide was biotinylated through amine group condensation across a polyethylene glycol spacer and then bound to the streptavidin-coated surface. Injection of the cellular sample caused an increase in the SPR signal due to the interaction between the EGF peptide and the membrane receptor expressed on the A431 cells. The selectivity and specificity of the sensor surface was confirmed through the competitive addition of free ligands to the analyte sample, which lead to a decrease in the SPR response [41]. The examples above show that SPR can be a versatile tool to resolve expression ratio of cell surface markers, distinguish cell phenotype and acquire cell-surface adhesion kinetics. In the next section, we will review the different methods used to assess cellular response to various stimulants.

Application of SPR in the analysis of cellular behavior under various stimuli

To assess cellular response upon stimulation, SPR was used to measure changes in morphological adherence of immobilized cells on the surface. These morphological alterations can be an indication of various intracellular or extracellular events of the cells of interest and; hence, analyzed based on respective applications. Conventional SPR, SPRi and long-range SPR (LRSPR) have each been used for assessing these cellular responses [15,17,42-44].

Prior to SPR cellular response analysis, cautionary measures need to be considered to prevent any impairment to the cellular function while recovery. Efforts in measuring reactions of nonadherent and adherent cells using SPR were led by Yanase *et al.* In this study, several techniques were considered and compared with recover cells and assess any potential functional impairments [17]. To assess nonadherent cells, multiple immobilization approaches were investigated. One method involves anchoring biocompatible probes to fix the cells on the biochip surface [17]. In order to anchor the nonadherent cells, biocompatible anchors for cell membranes (BAMs), and chemical linkers such as amino-alkanethiols and dithiobis[succinimydyl]proprionate (DSP) were tested. Amino-alkanethiols were able to bind to the cell membranes through electrostatic interactions and DSP formed an amide bond by reacting with primary amines. The SPRi results were similar in all of the above-mentioned fixation methods regardless of which anchoring probe was used (Figure 4). However, amino-alkanethiol was chosen over DSP and BAMs for further experiments because DSP binds nonspecifically to proteins in the incubation buffer and BAMs or other membrane anchors require additional sample preparation prior to analysis. As for adherent cells, the standard trypsinization procedure which utilizes trypsin (serine protease) as the cleavage enzyme was compared with vigorous pipetting at 4°C on standard cell culture dishes. In addition, recovery of cells from a floating state using a 3D construct of two biocompatible polymers, HydroCell(TM) and RepCell(TM), one of which is superhydrophilic and the other a temperature responsive polymer, was also assessed. Analyzing the cellular activity under the same stimuli using SPR concluded that

trypsinizing and scrapping cells at 4°C causes some impairment to the cellular function of human basophil and human B cells as shown with a lower SPR signal compared with the two methods employing the polymers. As a result, this work illustrates the potential of SPR as a clinical tool to study key cellular processes such as basophil histamine-release. In comparison to the more conventional method like ELISA for cell secretion analysis, SPR provides you with real-time measurements where ELISA requires an incubation period and labeling. Inherently, SPR will save your time and money.

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Figure 4. Angle of resonance response of anti-IgG binding to human basophil cells anchored on the surface by various molecules. Surface plasmon resonance imaging kinetic response of on-chip human basophil cells anchored by **(A)** cysteamine, **(B)** 8-amino-octanethiol, **(C)** DSP or **(D)** biocompatible anchors for cell membranes. The cellular response was monitored upon stimulation with a buffer containing or not containing anti-IgE antibodies. AR: Angle of resonance; DSP: Dithiobis[succinimydyl]proprionate.

In a different study, RBL-2H3 rat mast cells and PAM212 mouse keratinocytes were cultured on the biochip and morphological changes were assessed upon exposure to specific stimulants [15]. RBL-2H3 cells were exposed to dinitrophenol conjugated to HSA after sensitization with specific rat monoclonal anti-DNP IgE and this showed an increase in SPR signal. PAM212 cells showed a significant increase in SPR signal followed by a decrease after exposure to the EGF. Both SPR signals signify intracellular events occurring as a result of the stimulation that causes a distributional association or dissociation of proteins in the plasma membrane. As a result of the study, SPR was able to shed light on the underlying intracellular signaling events that are occurring upon stimulation or interaction with extracellular stimuli.

Single-cell-substrate interaction and cell-adhesion strength provide essential information about cell growth and detachment, cellular interaction with the extracellular matrix, and cell mobility [42]. SPRi offers an advantage for these applications mainly due to the real-time visualization of the sensor surface and the ability to select a region of interest for analysis [45]. Conventional SPR was developed into a SPRi sensor to allow for a higher-throughput biosensor screening [31,46-49], which further enables a more accurate single-cell analysis compared with an average change in RI corresponding to a large number of cells on the biochip area. Utilizing the extracellular osmotic pressure as the stimulus, cell-adhesion force is monitored on the surface of the biochip by its vertical displacement. The SPRi provided information about both the temporal and spatial resolution of the local movement of the cells, which is facilitated by the integrin-ECM linkages after being mechanically stimulated by the osmolarity of the extracellular matrix [42].

This advancement was very useful for cellular activity purposes because it allowed further understanding of the mechanism and localization of intracellular events occurring upon cellular activation [43]. The changes in SPRi signal indicate that the intracellular response to stimulation is not only limited to cell adhesion areas but also to changes occurring in specified locations inside the cell. For instance, as an extension to the above-mentioned study, the reaction of RBL-2H3 cells to antigen showed two different RI signals in the same cell; a rapid increase near the nucleus compared with a slower one near the cell membrane. The system was able to detect more than one reaction-type on the same biochip area, which is of great value for high-throughput screening in research and clinical applications. In addition, this platform was capable of

distinguishing activated cells from ones that are not activated prior to the stimulation with the antigen, which highlights the multiplexing capabilities of SPRi in cellular analysis [43,50].

In comparison to conventional SPR, LRSPR allows for an extended observation and localization of intracellular events due to the larger depth of penetration (1 μm) [51-55]. LRSPR occurs in the presence of multilayer dielectric [56] composed of different combination of materials, whereas SPR requires only a single dielectric. Both conventional SPR and LRSPR were compared in testing the cellular reaction of normal rat kidney epithelial cells to induced osmotic stress. It is well known that cell volume and structure play a major role in cell integrity and can mark different stages in cell morphology. The most noticeable difference observed upon the direct comparison was the presence of signature peaks or spikes in the conventional SPR response right after varying the buffer osmolarity. These immediate and transient responses were absent or less pronounced, when the same cellular response was monitored by LRSPR (Figure 5), which is mainly due to the difference in bulk sensitivities between the two techniques. Cellular events occurring at the cell membrane-surface junction are the most pronounced and dominating in the conventional SPR sensor response; hence, an immediate spike was observed. However, in LRSPR, these cellular events are concealed by other changes occurring in the remaining cellular organelles, which are located deeper in the evanescent field and further from the SPR surface [44]. These findings elucidate a more thorough understanding of the individual cellular events influencing the SPR signal using LRSPR.

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Figure 5. Conventional surface plasmon resonance and long-range surface plasmon resonance monitoring induced environmental changes to normal rat kidney cells. Surface plasmon resonance imaging kinetic response to a hypertonic solution exposed to confluent normal rat kidney cells by conventional SPR **(A)** and long-range SPR **(B)** that is provided by Dulbecco's phosphate-buffered saline^{++} buffer supplemented with sucrose to give a 25-mM solution (solid line) or a 50-mM (dashed line) solution. SP: Surface plasmon; SPR: Surface plasmon resonance.

SPR imaging ellipsometry (SPRIE) was developed to improve the selective study and imaging of cell-matrix adhesion properties and dynamics. This platform incorporated two components to improve the spatial resolution, an attenuated total reflection coupler and a null-type imaging ellipsometry for a better contrast. This new platform shows some advancement to SPRi because it allows for the use of short wavelengths without affecting the image contrast [57]. In this particular study, the adhesion properties and dynamics of various cell lines were investigated in real time using SPRIE. Cellular dynamics of dividing human umbilical vein endothelial cells, cell-cell communication and shear stress-induced dynamics were all monitored in a highly sensitive manner. The results showed that SPRIE is capable of providing a more detailed and sensitive understanding of the mechanism of cellular dynamics during cellular migration and division with a 1-mM spatial resolution [18].

SPR monitoring of cellular health upon exposure to harmful agents

Several groups have reported assessing cellular morphological variations upon exposure to various chemical agents using SPR. In one particular study, lipopolysaccharides (an endotoxin), sodium azide (a chemical toxin) and thrombin (a physiological agonist) were tested on human embryonic kidney-293 to assess cell viability [58]. Lipopolysaccharides are well known to cause cell death by triggering an inflammatory response [59], sodium azide is known to inhibit cellular

respiration [60] and thrombin is documented to cause contraction in the cells as it affects the cell integrity [61]. The cells were attached to the surface by poly- l -lysine, which is a cationic polymer that interacts with the polyanionic cell surfaces, and this in turn promotes cellular adhesion [61]. The SPR signals were correlated with phase contrast microscopy (Figure 6) for proper analysis of the cellular response as a result of exposure to these chemical and biological toxic agents. For example, as expected the SPR signal obtained after treatment with the lipopolysaccharides and sodium azide indicates the induction of membrane blebbing followed by cellular death and shrinkage of the cell body, respectively. Ultimately, this work revealed that SPR could be used as a reliable tool for assessing the cellular health upon exposure to various agents.

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Figure 6. Surface plasmon resonance response of cells upon stimulation with three separate biomolecular and chemical agents. (Left) Surface plasmon resonance signal change of HEK-293 cells upon exposure to **(A)** HBSS solution, **(B)** lipopolysaccharides, **(C)** sodium azide and **(D)** thrombin. (Right) Phase contrast micrographs taken at different time intervals after the injection of the described stimuli. Scale bar: 10μm. HBSS: HEPES-buffered salt solution; LPS: Lipopolysaccharide.

Expanding on this work, Maltais *et al.* showed interest in monitoring and quantifying programmed cell death events in a real-time and label-free manner [62]. To achieve this, they assessed the membrane alterations using SPR that occur during the process of apoptosis. Stimulation of the endothelial cell membrane death receptor (DR4-5) causes a complex intracellular signaling pathway characterized by a latency period where there are no signs of cell death, an execution phase and finally cell death. SPR analysis of the apoptotic events allowed the quantification of multiple parameters such as the duration and rate of the latency and execution periods. In addition, more studies were performed with other cell detachment or toxic trigger factors such as trypsin, sodium azide and 1% (w/v) sodium dodecyl sulfate and correlated with phase contrast microscopy to relate and confirm that morphological changes in cellular construct correspond to SPR signal changes. Traditional toxicity assays like lactate dehydrogenase rely on the use of labels and the analysis of an average response from many cells. However, SPR can assess cellular health label-free and real time. We foresee, in the future, SPRi systems will be employed for this type of test more than SPR systems as they will enable us to visualize and measure kinetically a single-cell activity, label-free in real-time.

Using SPR to monitor bacterial growth & removal from surfaces

Bacterial contamination and growth constitute a main concern in industrial and environmental settings; hence, current research is directed toward finding reliable detection techniques and solutions for the removal of pathogenic species [63,64]. SPR has gained considerable popularity in this area as a reliable source of information as it offers highly sensitive capabilities and avoids the labeling requirement, high reagent costs and long analysis time imposed by other detection strategies [64-67]. In research performed by Abadian *et al.* , the activity of *E. coli* and *P. aeruginosa* was visualized using SPRi. Bacterial attachment, movement, growth to a biofilm and removal were studied using this biosensor revealing significant information about each process. In the initial set of experiments as shown in (Figure 7), the growth of green protein fluorescent (GFP)- *E. coli* was monitored at different time intervals (6 min, 1 and 6 h) while incubated on the surface of the chip inside a rectangular polymer chamber. The sensing platform incorporated

microfluidic channels composed of rectangular polydimethylsiloxane chambers to separate bacterial samples from each other and the environment. In one chamber, GFP- *E. coli* was added along with LB (lysogeny broth) growth media and in the second one only LB was added as a control. The appearance of bright spots in the SPRi difference images (Figure 7B) of the bacterial chamber is due to biomass accumulation on the surface, which signifies the growth of bacteria. The control chamber remained dark after 6 min (Figure 7A) until the appearance of bright features on the gold surface (Figure 7C & E), and this is due to the evaporation of growth media from the chamber. The bright spots in the difference image are a result of the difference in RI between the growth media and air. Finally, the SPRi images of both control and sample chambers were correlated with fluorescent images (Figure 7G,H) and as expected fluorescent cells were visualized in the bacterial chamber only. This study revealed both kinetic information about bacterial adherence and growth and spatial information about the biofilm formation [28].

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Figure 7. A study of biofilm formation using a custom microfluidic surface plasmon resonance imaging platform. (Top) A schematic representation of the surface plasmon resonance imaging experimental setup for measuring *E. coli* biofilm growth. (Bottom) Surface plasmon resonance imaging images of the blank chamber after **(A)** 6 min, **(C)** 1 h and **(E)** 6 h; GFP-labeled *E. coli* -filled chamber after **(B)** 6 min, **(D)** 1 h and **(F)** 6 h. Fluorescence images of the blank chamber **(G)** and of the GFP-labeled *E. coli* -filled chamber **(H)** . LB: Lysogeny broth; PDMS: Polydimethylsiloxane.

Monitoring of biofilm formation was followed by another set of experiments by the same group focusing on the removal of mature biofilms from the sensor surface [28]. GFP- *E. coli* was allowed to attach and form a robust biofilm on the sensor surface for 24 h at 37°C. The biofilm was then subjected to deionized water for 11 min followed by ethanol for 5 min flowing at a rate of 2 ml/min inside the SPRi apparatus to allow complete removal of the biofilm. Images were taken at different time intervals (Figure 8) and after a number of repeated experiments, the biofilm removal appears to begin at the highest upstream point of fluid flow and continues downward in a complete manner without any cellular material left on the sensor surface. In conclusion, this work introduces for the first time a highly sensitive technique to study bacterial properties on surfaces in a label-free manner. The SPRi gold surface is ideal for carrying out further experiments for characterizing bacterial adhesion properties, which will serve in medical, industrial and environmental applications.

FIGURE 8 IS OMITTED FROM THIS FORMATTED DOCUMENT.

Figure 8. Surface plasmon resonance imaging biophysical anlaysis of biofilm removal. **(A)** Surface plasmon resonance imaging difference image of the initial phase of the experiment. Monitoring the removal of the biofilm after flowing deionized water after **(B)** 3.7, **(C)** 5.8 and **(D)** 7.5 min. **(E)** Ethanol was then flowed over the surface for 5 min, and rinsing with deionized water followed this. **(F)** No change is observed after 25 mM phosphate-buffered saline (pH 7) was flowed for 8 min, followed by ethanol for 7 min and deionized water for 5 min. Arrows indicate direction of fluid flow.

Challenges & limitations

Cellular analyses using SPR is still in its infancy stage, before we can observe rapid growth for this type of application several challenges and limitations need to be addressed. For example, the SPR sensing range only spans 300 nm from the sensor surface and the diameter of cells range between 1 and 30 μm, so inherently with SPR you are limited to only detecting events occurring

around a small portion of the cell. Therefore, you can only attain qualitative analysis. Alternatively, LRSPR was introduced as it extends the detection range up to 1 μm. However, one potential setback for sensing using an imaging configuration is the loss of image resolution due to the extended propagation length. In addition, accessibility to LRSPR currently is limited as there are no commercially available vendors for it in the market. Perhaps, to take advantage of the sensitivity of the current commercially available SPR systems would be an alternative approach. For example, the possibility of exploring more planar surfaces or the use of bifunctional linkers that rely on host-guest interactions chemistry that could potentially allow for the cell to reside closer to the sensor surface for improved analysis. Another challenge is maintenance of cell integrity during the immobilization and handling process. Direct immobilization of cells onto the biochip could cause damage, if care during the handling process is not taken into consideration. As a result, majority of work reported with cells and SPR involve binding of cells to biochips that have been prefunctionalized with a capture ligand (i.e., antibodies). Finally, another aspect that could potentially limit cell-SPR application would be clogging in the fluidics as certain instruments can only tolerate ≤1 μm particulates.

Conclusion

In this review, we demonstrate the applicability of the SPR platform in assessing single-cell dynamics by minimizing false/positive results associated with labels and mixed cell populations. Specific whole-cell detection is investigated through multiple surface chemistries of immobilized ligands such as DNA, antibodies and protein peptides. This sensitive and selective detection of living cells allows for the utilization of SPR toward many applications and especially for cell sorting. The SPR sensor surface enables real-time analysis of cellular activity upon exposure to external stimuli for both adherent and nonadherent cells. Analysis upon stimulation of cells leads to the localization of various intracellular events and cellular interaction with the extracellular matrix. This is complemented with kinetic information of individual cellular processes, which helps in improving the understanding of cellular adhesion properties, alterations in cellular morphology, biofilm development and removal. In the future, we foresee the advancement of the SPR platform to include a wide range of biomedical applications serving to better analyze cellular activity.

Future perspective

Analysis of cellular events using SPR is still new and requires some fine tuning; however, we foresee that it will lead to new applied life sciences discoveries that could potentially impact unmet biomedical solutions. In addition, we foresee that in the future that the integration of SPR with other existing technologies like fluorescence and Raman will provide unique platforms that can potentially overcome some of the existing limitations with cellular analysis using SPR alone. For example, Raman microscopy [68] is used to analyze the chemical fingerprint of cells. In combination with SPR, not only you are able to attain chemical fingerprinting analysis along with surface reaction kinetics but also the marriage of these technologies can generate a stronger unified analytical tool and expand their capabilities. This strength was illustrated in the works of Liu *et al.* , where they integrated LRSPR with surface-enhanced Raman scattering (SERS) [69,70] and observed 15-times stronger Raman signal in comparison to conventional SPR. The next step is to assess whether SPR-SERS can detect Raman-inactive molecules. Currently, there

is only a handful of manuscripts [71-73] that describe the setup of SPR with SERS and their potential impact. Another promising amalgamation is SPR with fluorescence. The combination of the two technologies will allow for simultaneous SPR and surface plasmon-enhanced fluorescence live cell analysis. One of the drawback with SPR is the inability to measure the whole-cell response; however, combined with surface plasmon-enhanced fluorescence, Chabot *et al.* [74] were able to identify the precise molecular mechanism liable to the SPR response as a result of the introduction of a stimulant to cells. Finally, as SPR evolves with other existing techniques, cellular responses and mechanisms will be easily elucidated and advance solving outstanding problems in the biomedical field.

Executive summary

- Single-cell analysis is needed to understand cellular dynamics that are masked by the inherent heterogeneity of complex populations.
- Surface plasmon resonance (SPR) is a label-free, real-time biosensing tool for the sensitive and selective detection and quantification of biomolecular interactions.
- Application of SPR in the analysis of cell receptor-ligand interactions:
    - Selective detection of live cells by antibodies immobilized on the SPR surface;
    - Live cell capture and controlled release by complex DNA and antibody constructs;
    - Detection of cellular interaction with peptides immobilized on the gold surface.
- Application of SPR in the analysis of cellular behavior under various stimuli:
    - Cellular activity of both adherent and nonadherent cells can be monitored on the SPR sensor surface upon exposure to various stimulants;
    - Insight into intracellular-signaling events, cellular interactions with the extracellular matrix and adhesion properties of different cells;
    - Long-range SPR and SPR imaging ellipsometry (SPRIE) both advance the sensing capabilities of SPR by providing a more thorough analysis of the intracellular events over various cellular organelles due to the larger depth of penetration (1 mm) and better spatial resolution.
- SPR monitoring of cellular health upon exposure to harmful agents:
    - Morphological changes in cells such as shrinkage can be monitored upon exposure to chemical agents on the sensor surface and this is paired with kinetic data of individual cellular processes after the exposure.
- Using SPR to monitor bacterial growth and removal from surfaces:
    - SPRi is shown to be effective as a sensitive technique to monitor bacterial adherence and growth at different time intervals on the biosensor surface yielding valuable information for medical and environmental applications.

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