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S.I. : Biomaterials - Engineering Cell Behavior

The Convergence of Cell-Based Surface Plasmon Resonance and Biomaterials: The Future of Quantifying Bio-molecular Interactions—A Review

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Abstract—Cell biology is driven by complex networks of biomolecular interactions. Characterizing the kinetic and thermodynamic properties of these interactions is crucial to understanding their role in different physiological processes. Surface plasmon resonance (SPR)-based approaches have become a key tool in quantifying biomolecular interactions, however conventional approaches require isolating the interacting components from the cellular system. Cell-based SPR approaches have recently emerged, promising to enable precise measurements of biomolecular interactions within their normal biological context. Two major approaches have been developed, offering their own advantages and limitations. These approaches currently lack a systematic exploration of 'best practices' like those existing for traditional SPR experiments. Toward this end, we describe the two major approaches, and identify the experimental parameters that require exploration, and discuss the experimental considerations constraining the optimization of each. In particular, we discuss the requirements of future biomaterial development needed to advance the cell-based SPR technique.

Keywords—Biomolecular interactions, Surface plasmon resonance (SPR), Cell-based SPR, Systems biology.

INTRODUCTION

Systems biology is a growing field that incorporates biological measurements with computational modeling to uncover new understandings of biological systems, measurements which require the use of advanced biomaterials to capture biologically-accurate conditions.[11,15] Different systems biology studies explore physiological systems under normal and pathological conditions. Computational systems biology approaches have been applied to describe endothelial cell apoptosis signaling pathways,[118] investigate vascular endothelial growth factor (VEGF) family activity,[65] explore and design better pro-angiogenic therapies,[66] and predict cell response from the protein–protein interactions occurring within a cell.[113] Thus, systems biology has advanced knowledge of the underpinning mechanisms behind cell processes.

Despite this progress, deterministic models based on mass action kinetics have been limited by a lack of quantitative data on biomolecular signaling and interactions. Mass action kinetics models are defined by both the amount of species (concentrations), and the probability of these species interacting (i.e. binding kinetics). Therefore, data needed to parameterize such models are both protein concentrations and protein–protein interaction kinetics. Although there is a plethora of qualitative data available on protein expression (e.g., Western blots) and protein–protein interactions (e.g., co-immunoprecipitation), there is a need to move from *qualitative* to *quantitative* characterizations of biomolecular interactions. To address the quantitative data limitation, systems biology researchers are developing new assays to measure protein concentrations[9,18,51] and build databases[71,74] that aggregate data and provide researchers with the

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information needed to build computational models. Indeed, we and others have led efforts to quantify protein concentrations on cell membranes[16,18,48–51,113]; thus supplying data to computationally model vascular signaling, which is critical to advance engineering goals of vascularizing tissues.[29,69,113–115]

However, current approaches for measuring binding kinetics for biomolecular interactions involving membrane-bound proteins are performed using recombinant versions or the full protein extracted from the membrane.[23,78] Such approaches, therefore, measure protein–protein binding outside of their biological environment, such as within a cell membrane, which can result in different protein confirmations. Since protein conformation differences can impact their binding and signaling abilities,[60,100] performing these measurements outside of their normal biological context could produce results that poorly reflect the actual dynamics in biological systems.

However, there are currently few experimental approaches to measure biomolecular kinetics in biologically native conditions. Recently, the surface plasmon resonance (SPR)-based biosensor approach has been expanded for use with whole cell samples instead of purified protein samples.[83,91] Cell-based SPR approaches offer the promise of high-throughput quantification of biomolecular interaction kinetics and affinities under biologically native conditions. While recent studies have measured membrane-bound protein–protein kinetics, there remain several critical questions unanswered and unexplored regarding assay optimization and best practices. We overview the different approaches developed to adapt SPR biosensor assays to measuring kinetics on whole cells, describe the key experimental conditions that ultimately require optimization, and layout a general guide towards establishing best practices for the major variants of cell-based SPR.

MEASURING BIOMOLECULAR KINETICS *VIA* SPR

Kinetic and Thermodynamic Properties Characterize Biomolecular Interactions

Biomolecular interaction dynamics are best characterized by: (1) binding kinetics and (2) binding affinities.[82] The binding kinetics represent the *rate* at which the proteins bind and dissociate. In a 1:1 protein interaction, the kinetics are characterized by two quantifiable properties: the *association constant* k_{on} describes the rate that two proteins bind to form a complex; the *dissociation constant* k_{off}, in turn, describes the rate this complex dissociates, back to the unbound molecues.[53] The binding affinity describes the 'strength' of the protein interaction.[80] Conventionally, binding affinities are expressed as the equilibrium dissociation constant K_D; the higher the K_D value, the lower the binding *affinity*. Conveniently, K_D can be expressed in terms of the kinetic rate constants (Eq. 1). The binding affinities and kinetics reflect intrinsic structural and chemical properties of the involved molecules, and are therefore altered by post-translational protein modifications.[71]

$$K_D = \frac{k_{off}}{k_{on}} \tag{1}$$

SPR to Identify and Measure Biomolecular Binding Kinetics

The SPR-based assay is an ideal approach for identifying and measuring kinetic rate parameters for biomolecular interactions, like between growth factors and their receptors. SPR-based biosensors like the BIAcore[81] detect protein–protein interactions utilizing an optical phenomenon that is sensitive to small changes in mass near the sensor surface.[8,99] By coupling a target protein on the sensor surface, binding kinetics and affinities can be measured by flowing the protein analyte through a flow channel over the sensor surface (Fig. 1a) and recording the mass change over time while analyte binds and unbinds the target protein.[21,79,80] The binding kinetics and affinities are then determined by fitting these data to mathematical equations that represent specific chemical binding models, as described thoroughly in several excellent reviews.[45,77,79] Furthermore, SPR-based biosensors are capable of probing one analyte against multiple targets simultaneously, enabling faster measurements of different protein–protein pairs.[33] Therefore, SPR-based assays have proven an ideal approach for measuring binding kinetic parameters for biomolecular interactions.

SPR is a Label-Free, Highly Sensitive, and Cost-Effective Approach to Measure Biomolecular Interactions in Real Time

SPR-based approaches have several fundamental advantages over other existing affinity and kinetics assays. Several of these assays have been reviewed extensively by others,[35,40,52] and include: fluorescence-based, radiolabeling, and enzyme immunoassays.[23,26,52,107] For measuring binding kinetics, SPR has four major advantages: (1) SPR is a label-free

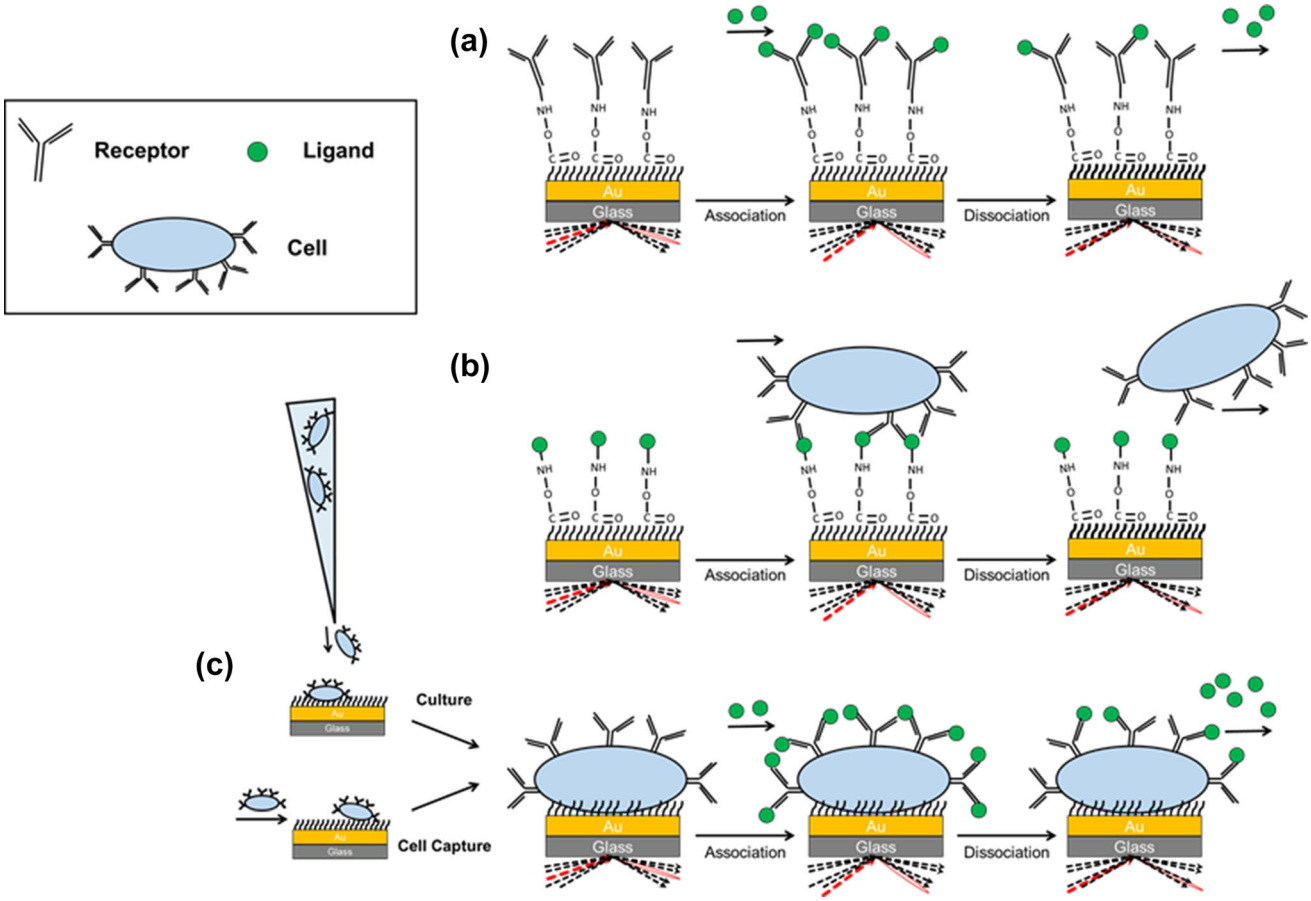

FIGURE 1. SPR approaches for kinetics and affinity measurements. (a) Traditional SPR compared to the cell-based SPR approaches, (b) injected cell analysis (ICA) and (c) immobilized target cell (ITC) appraches.

technique, unlike other approaches, which require coupling an additional reporting label, such as radioactive compounds or fluorescent tags, to one or both proteins. Such tags, therefore, can interfere with protein–protein binding.[88] (2) SPR biosensors detect binding in real-time: protein association and dissociation responses are detected as they occur, allowing straightforward binding kinetic measurements.[33,77] (3) SPR detects interactions with high sensitivity and can therefore measure binding kinetic and affinity constants to higher precision than other techniques. For example, binding affinities (K_D) on the scale of picomolar (pM) can be measured using SPR, while fluorescence, absorption assays, and calorimetry assays measure binding affinity on the μM–mM scale.[23,52] (4) SPR requires relatively small sample quantities, using protein solution volumes of 10–20 μL per sample,[52,69,96] whereas calorimetry and absorption assays require mL quantities.[52,75] Altogether, SPR offers a reliable technique to accurately characterize binding kinetics and affinities of biomolecular interactions.

Conventional SPR Limited to Characterizing Biomolecular Interactions Outside Their Native Environments

Conventional SPR-based approaches have been primarily limited to measuring biomolecular interactions in isolation, outside of their biological context. For membrane-bound proteins, SPR experiments are typically performed using recombinant partial version of membrane receptors that often include only the extracellular domains, rather than including transmembrane domains.[76] Measurements with partial proteins can produce non-physiologically relevant results, because binding is often regulated by conformational changes in receptor subunits.[119] Additionally, the membrane-bound protein is typically covalently bound to the sensor surface *via* amine coupling, creating a physiologically inaccurate system, since the membrane protein environment should facilitate interactions with cholesterols, lipids, and other membrane-bound proteins.[87] An innovative workaround to this limitation is to perform these measurements on

nanodiscs—self assembled lipid-bilayers—containing the target protein in an environment mimicking the cell membrane.[98,102] However, nanodiscs do not entirely mimic the cell membrane composition, as they lack cholesterol and other membrane proteins. These differences are critical, as studies have demonstrated that membrane protein binding properties can vary depending on membrane composition, such as the cholesterol concentrations.[37,58] Furthermore, purified or recombinant membrane proteins will lack the post-translational modifications, like N-linked glycosylation, which have been shown to alter binding properties.[103]

An additional improvement on these existing approaches would be to perform SPR measurements with actual cells. Cell-based SPR is an emerging technique that combines the experimental benefits of SPR-based bioassays with the ability to measure interactions on receptors within actual cell membranes. Optimizing these approaches, however, to obtain useful chemical kinetic and affinities remains unexplored and will require significant advancements in biomaterials to ensure existing SPR biosensors provide ideal conditions for use with whole cells.

CELL-BASED SPR APPROACHES

Two major approaches have been developed to adapt SPR approaches, using standard SPR instrumental setups, to measure interactions with live cells by substituting the cells for either: the analyte, by flowing the target cell through the system, referred here as *the Injected Cell Analyte (ICA)* approach (Fig. 1b)—or the immobilized/target protein—i.e. the protein immobilized to the sensor surface, called here the *Immobilized Target Cell (ITC)* approach (Fig. 1c).

Immobilized Target Cell Approach

The *Immobilized Target Cell (ITC)* approach monitors injected ligand binding to membrane or surface proteins on cells immobilized to the SPR sensor chip (Fig. 1c). This approach provides the advantage of directly measuring the equilibrium dissociation constant K_D, because known concentrations of analytes are injected before each experiment. Therefore, an ITC approach allows measuring kinetic rate constants directly. Moreover, the binding kinetic constants measured will reflect the *effective* binding between the ligand and the target receptor while incorporating the effects introduced by other modifications, such as differing membrane composition and non-specific ligand-membrane effects. Nevertheless, the ITC approach has disadvantages. First, due to inherent limitations of SPR,[99] the short penetration depth of the evanescent field cannot detect the whole cell and the physical binding activity. This leads to smaller apparent response levels as binding is only detected to the part of the cell that is in the evanescent field (about 300–400 nm) above the gold sensor chip surface. However, a novel SPR system that uses near-infrared incident light—instead of visible light, as used in conventional SPR systems—generate evanescent fields that extend 10 μm, vastly extending the detection range. These Fourier transform infrared spectroscopy (FTIR) SPR systems, therefore, would enable detecting activity across the entire cell,[122] and has already been used to monitor membrane composition changes in HeLa cells and detect endocytic processes in human melanoma cells.[120,122] Additionally, researchers recently demonstrated that the evanescent field depths could be extended to *2* mm using a graphene-based biosensor in place of the conventional gold sensors, and used the expanded signal depth to study drug-responses in whole cancer cells.[111] Additionally, attached cells can detach from the surface more readily than covalently bound receptors as found in traditional plasmon resonance-based approach. Both differences introduce challenges that require the selection of optimal flow rate conditions and biomaterial choices for the sensor surface.

A Need for Biomaterials: Maximizing Cell-Sensor Adhesion via Sensor Coating and Functionalization

The adhesive strength—i.e. the attachment force between the cells and the surface in resistance to shear—of the chosen surface material is key to designing a cell-based SPR study using an ITC approach. A surface material with a weak adhesive strength will weakly immobilize cells and result in cell detachment when buffer or analytes are injected over the channel surface. Conversely, adhesive strength that is too strong may cause cells to spread abnormally.[73] With the ITC approach, there are typically two methods implemented to adhere cells to the surface: directly culturing cells on the sensor surface *via* overnight incubation,[17,119] or flowing cells onto the sensor surface.[95] In both cases, however, adhesion of cells can be greatly affected by surface coating. Typically, a short-chain surface such as a derivatized alkanethiol is used as the backbone of the surface to ensure that the captured cells are close to the sensor chip surface to optimize detection. Hydrogels such as dextran are not recommended because they usually extend 100 nm from the sensor chip surface, which would cause more of the cell to not be in the evanescent field. The chip with the short chain alkanethiol groups that also contain some carboxyl groups are typically derivatized

with a biomaterial to provide an adhesion matrix for the cells. Therefore, cell adhesion to the chosen material must be tested. For example, cells adhered to poly-L-lysine (PLL) coated surfaces can flatten against the surface due to the interaction between positively-charged poly-L-lysine and anionic cell membrane.[73] Identifying the best material may be daunting when one couples the need for optimal adhesive strength with the many immobilization material choices. Amongst different approaches, some common ones include high-affinity biomolecules, like antibodies, engineered peptides, and aptamers[5,6,39,57,110,121]; extracellular matrix proteins, like fibronectin, collagen, and laminin; or cationic molecules, like lipids,[67] polymers,[62,73,94] and peptides.[46,68] When choosing adhesion molecules, one ought to consider the interactions between chosen molecules and membrane proteins, such as coating a sensor with an integral protein membrane like CD31.[7] The approach and the adhesive molecules used to target cells should not compromise the need for optimal adhesive strength. There are several guides in literature for choosing optimal materials. For example, several molecules have been optimized for high cell binding specificity in the drug delivery field.[2,24,84,89] The biomaterial porosity should also be considered in context of the analyte molecular size, to prevent the injected analytes to leech into the surface, registering falsely as binding signal. Likewise, the chosen biomaterial should not incorporate chemical functional groups that resemble the analyte binding target sites. The cell patterning,[36] affinity microfluidics[47] and biomaterials fields[20] also offer immobilization material guidelines.[4] In these fields, extracellular microenvironment mimics have been engineered to enable optimal cell residence and honing.[1,70,90] Altogether, it is critical to identify the optimal surface material, which should facilitate cell immobilization with good adhesive strength while being specific to the cell and receptor biology.

Reducing Non-specific Binding and Preserving Cell Surface Receptors Contributes to the Selection of Cell Immobilization Approaches

One important parameter to control in SPR experiments is non-specific binding: the interactions between analytes and non-targeted molecules and/or the sensor surface.[44] Traditional SPR-based kinetics approaches are prone to signal associated with non-specific interaction[38] which requires reference correction. This consideration carries over to ITC approach-based cell-based SPR. The incorporation of a reference channel—i.e. a separate sensor channel where the ligands have no specific interaction target—is the standard approach to obtaining a background reference signal, which is subsequently subtracted as correction.[38,69,97] Selecting a background reference target, however, is challenging (as described previously[38]), and deciding on an appropriate reference in cell-based SPR is dependent on the ITC sub-approach taken. When immobilizing cells on the chip *via* direct culturing on the chip,[43,46] it is difficult to separate the experimental side of the chip from the reference side of the chip since culturing different groups of cells on the same sensor chip can be problematic. When cells are immobilized onto the chip by injecting the cells over the sensor surface, a reference can be easily achieved. A reference channel is ideally created by immobilizing non-active cells that are not expressed with analyte receptor at a surface density similar to that achieved for the active cells immobilized onto the sample channel. Alternatively, a reference channel could be left as the surface matrix backbone itself. For adherent cells or cells from tissue, a single cell suspension can be obtained *via* enzymatic dissociation from flasks or tissue, respectively. This must be tested, because enzymatic agents may be disrupt the membrane proteins to be studied *via* SPR.[18] Before cell-based SPR-based approaches can be utilized more commonly, therefore, the question of an ideal background reference signal source must be answered.

Minimizing Rebinding Effects Through an Optimized Cell Density

An ideal cell density for studying kinetics should result in a measurable increase in SPR signal compared to the background signal while minimizing rebinding effects. If the sensor surface cell density is too low, then injected analyte may result in a low binding signal, whereby differentiating the true binding signal from the background, non-specific signal becomes increasingly difficult.[72] Conversely, injecting over a high-density surface can result in target-rebinding effects and promote significant non-specific cell attachment.[83] In each case, the unwanted effects will interfere with measuring the true binding kinetics. Another consideration is the receptor density on the surface of the cells and the molecular weight of the analyte that binds to the cells. If the cells are enriched with receptor, then a lower cell density can potentially be used. In addition, for large analytes (> 100 kDa), a lower density can also be used in comparison to a smaller analyte, as the SPR signal is sensitive to the total mass that binds to the surface. While appropriate immobilized protein ranges have been determined for traditional SPR experiments,[25,41,42] no comparable systematic study has been performed for cell-based SPR approaches. Researchers have investigated this indirectly, by varying the cell concentration range they inject to coat the

sensor surface, but these covered a narrow window (600 cells/mL[46] to 1600 cells/mL[119]), and do not provide researchers with guidelines for *surface densities*. Future work, therefore, is required to systematically test cell injection concentrations to determine ranges that optimize the detected signal while minimizing the negative effect of non-specific cell adhesion and rebinding effects.

Optimizing Analyte Flow Rates to Minimize Cell Shear Stress and Avoid Mass Transport Limiting Conditions

The analyte flow rate—the rate at which analyte is injected through the microfluidic system—is already an important optimization parameter in traditional SPR experiments,[33,41,106] and takes an additional importance for experiments injecting across captured cells. Flow rate serves as a critical element in fluid dynamics, and many biological processes take place in solution.[13] Analyte flow rates have previously been optimized to be fast enough to avoid mass transport limitation (MTL) effects.[55,97,101] But because shear stress is proportional to the flow rate,[85] setting the flow rate arbitrarily high could result in cells detaching from the sensor chip.[63] Therefore, flow rates must be optimized to be sufficiently high as to avoid MTL effects–which distort analyte :receptor binding kinetic measurements[81] –while minimizing the shear stress thus minimizing cell detachment rate. Analyte injection flow rates have been explored across a narrow flow rate range—50 to 20 μl/min—and chosen apparently arbitrarily.[43,119] A systemic study is required to establish criteria to optimize flow rate to minimize MTL effects while reducing cell shear stress.

Optimizing Sensor Regeneration Conditions to Minimize Cell Loss

In a traditional SPR analysis, five (5) concentrations of analyte are injected over the immobilized target that span a concentration range centered around the interaction affinity. If the rate of dissociation is slow (i.e., signal does not decay back to the starting baseline in 10 min), a regeneration solution is injected that disrupts the interaction between the analyte and target and returns the baseline back to the original starting value. If the target is covalently immobilized, the surface is regenerated back to free target and another analyte concentration can be injected. However, if the target is captured *via* a non-covalent means, the target is removed from the surface along with the analyte and would need to be reloaded for each analyte concentration. In the case of the ITC approach, the surface would be regenerated with a solution that would remove the cells from the surface along with the analyte, but the cells would then need to be recaptured for each analyte concentration. This approach would consume a large quantity of cells and it can be very difficult to remove all the bound cells from the chip surface. Alternatively, there is a different tactic that can be implemented instead of regenerating between each analyte concentration. This approach is called a kinetic titration whereby analyte is injected sequentially from low to high concentration without regenerating between injections.[22] This option is very attractive because it eliminates the need for regeneration, which would save on sample consumption and time, which is an important consideration for cell-based SPR.

Injected Cell Analyte Approach

The second general approach currently utilized in cell-based SPR is the *Injected Cell Analyte (ICA)* approach. This is opposite to the ITC approach in that the target cell is injected in place of the analyte protein over the immobilized target receptor. In the ICA approach: (1) the interactant to the cells—e.g. growth factors like VEGFA—are immobilized instead of the cells to the sensor surface. (2) Cells are injected across the immobilized ligand. (3) The surface is 'regenerated' to remove the bound cells from the surface before re-injecting at a different cell concentration (Fig. 1b).[32] While the protocols related to ligand immobilization and regeneration are well-established by traditional SPR analysis,[106] the use of cells as the analyte has its advantages and limitations. Both ligand immobilization and regeneration steps for cell-as-analyte approach can be adapted from traditional SPR techniques. The main drawback of this tactic is that since a molar concentration of cells cannot be determined, an association rate constant cannot be calculated as it is a function of molarity and time. However, qualitative information can still be learned from this approach. In addition, the number of regeneration cycles can be limited due to the potential loss of cell binding capacity.[91] Cell debris may affect SPR signal if the regeneration approach is not thorough. Like the ITC approach, several experimental conditions require optimization to ensure useful binding parameters are obtained.

Optimizing Cell Injection Flow Rates and Concentrations to Minimize MTL Effects and Maximize Response Signal

As in the ITC approach, the quality of the obtained data for the injected cell analyte approach is dependent on optimizing the cell injection flow rate in order to minimize mass transport limit effects. At high injection flow rates, the bulk flow concentration is higher than

the cell concentrations at the binding surface. Analyte depletion during association phase can be induced at the surface. If the bulk concentration is lower than the cell concentrations at the binding surface due to a low cell injection flow rate, a retention zone can be formed during dissociation phase.[101] With both conditions, the SPR signal will be altered, exhibiting slower binding and unbinding curve.[101] There is a need, therefore, for a systematic study of the optimal injection flow rate. However, no such systematic study has established an optimal cell injection flow rate range. Previous studies using this approach having used a wide range of rates, from 3[105] to 70 μl/min,[72] but no research has determined a protocol that optimizes these rates for specific cell types. Future studies, therefore, are needed to identify the ideal conditions.

Another major challenge in cell-based SPR is identifying the cell concentrations injected through the system, since both cell density and size ultimately impact viscosity and flow resistance. These effects have been observed in therapeutic fields, where the size and concentration of red blood cells alter blood viscosity.[54,63,85] High RBC concentrations, for example, increase blood viscosity and impair drug delivery.[34] These factors, therefore, will influence whether injected cells will effectively reach the sensor surface to bind immobilized target proteins and produce a signal. In cell-based SPR, an ideal cell concentration is the cell concentration that can produce a reliable signal. The reliability is determined by how easily we can differentiate specific binding signals from non-specific binding signals.[72] A higher cell concentration can produce a higher difference between ligand-receptor binding induced signal and background signal, yet a high cell concentration can lead to higher viscosity, causing a clog in the SPR system. Some early work has begun investigating the importance of injected cell concentrations in such studies: the relationship between binding rate and cell concentration were described as an exponential curve in a red blood cell binding study.[91] Further work is needed to determine the optimal cell concentrations for different cell sizes.

The Future of Cell-Based SPR

Cell-based SPR has been used to characterize the interactions between ligands and membrane protein receptors, and these membrane proteins are important for biological processes and are linked with certain diseases.[87] Understanding these interactions is critical for drug development. For instance, cell-based SPR can be performed to obtain the binding affinity and study dosage-dependent responses (e.g. anti-TNF agents[83]). In addition, cell-based SPR offers the opportunity to obtain biological signals triggered by agonists and antagonists. Cell-based SPR allows for the evaluation of pharmacodynamic parameters and for the prediction of the potency of new drugs.[59] Cell-based SPR can advance computational models of complex biological systems by enabling high-precision measurements of ligand:receptor kinetics that better reflect biological reality. Computational models serve as powerful tools to study complex biological systems,[118] because physiologically-relevant phenomena—such as tumor metastasis, wound-healing, or immune reactions—emerge from many cell-level interactions.[10,12,15,28] Modeling cell signaling pathways—whereby ligands bind membrane-bound receptors to trigger interwoven signaling networks to modulate cell activity—has provided insight into several growth factor-receptor families known to mediate physiologic and pathological processes,[19,28–31] including epidermal growth factors (EGFs),[116] fibroblast growth factors (FGFs),[27] platelet-derived growth factors (PDGFs),[86] and vascular endothelial growth factors (VEGFs).[64,66,92,93,104,108,114]

Such models are often constructed using the law of mass action, where an interaction rate is proportional to the interacting species' concentration and their underlying kinetics[3,28,56,69,112,117]; their predictive power is therefore limited by how accurately the experimental measurements of binding kinetics reflect biological reality. Traditional SPR assays rely on measuring protein–protein interactions removed from biological systems; e.g. VEGF-A:VEGFR and PDGF:PDGFR ligand:receptor interaction kinetics are measured by observing the ligand binding a recombinant receptor protein representing the extracellular portion only.[61,69,109] These experimental models, therefore, are limited because they cannot reflect factors that modulate ligand binding, such as membrane composition[58] and post-translational modifications (e.g. receptor protein n-glycosylation[14]). By measuring these interactions under more biologically comparable conditions, we can construct more accurate, useful models. Cell-based SPR is well-suited for these measurements by enabling highly sensitive kinetic measurements of the interactions between proteins and native cell membranes in a label-free environment.

Cell-based SPR achieves the measurement of protein–protein interactions within a biologically native environment. Both approaches—the immobilized target cell and injected cell analyte approaches—offer advantages towards obtaining biologically-representative parameters for computational modeling. The ITC approach allows measure binding affinities and kinetic parameters using the mathematical fitting approaches used in conventional SPR but requires careful optimization to ensure stable cell adhesion across experi-

ments. The ICA approach allows conventional chemical coupling techniques to immobilize target proteins to cell sensors, but injected cells face significant mass transport limitations due to cell size that require careful flow rate and cell concentration optimization to reduce. The ICA approach, therefore, may be better applied for small cell types, like with bacteria, while the ITC approach may be a better choice for cell sizes too large to effective flow as analyte. To enhance the outcomes of cell-based SPR and establish a standard procedure, each critical parameter should be optimized and a standard for assigning values to these parameters should be established.

Conclusions

The next steps should be to establish the optimal experimental conditions and standards of the cell-based SPR procedures. Although several different studies investigating living cell reactions in response to stimuli have been carried out using cell-based SPR approaches, there are no ''best practices'' for cell-based SPR throughout the literature. Experiments need to be performed to optimize critical parameters, such as cell density, ligand flow rate, and cell capture surface in the Immobilized Target Cell approach, as well as both cell concentration and cell flow rate in the Injected Cell Analyte approach. Developing a framework to optimize the key experimental parameters in cell-based SPR, can help researchers perform experiments in a more effective and meaningful manner. By establishing these optimal conditions, we can also better understand the effects of these parameters on the binding kinetics.

Cell-based SPR has proven to be a powerful tool to study both ligand-receptor binding and its subsequent signaling pathways in each study. An optimized method to perform cell-based SPR is necessary to ensure the meaningfulness of the outcome and expedite the applications of cell-based SPR. Regardless of the challenges that it may face, cell-based SPR has the capability of monitoring the dynamic changes at the binding site and cellular changes in a real-time and label-free setting. The advantageous capabilities of cell-based SPR can result in scientific breakthroughs for brain therapy and enhancements in novel therapeutics.

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