MEASURING CELLULAR ACTIVITY USING SURFACE PLASMON RESONANCE

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INTRODUCTION

Environmental and biomedical research are pushing towards the development of biosensors able to rapidly determine the activity of a biomolecule, such as an agonist or a toxin. Most of the research in these fields is in the development of specific biosensors, where the detector surface is functionalized in order to detect the presence of a specific target[1]. However, there is a need for broad-range biosensors, where the focus is to detect biomolecules involved in a wide variety of biological signals[2]. Living cell sensors fall into this category, measuring cellular signals following specific activation through numerous agents.

Among the optical detection methods for living cell sensing, surface plasmon resonance (SPR) is one of the most promising candidates for non-invasive detection without the need for labeling[3]. A surface plasmon is a charge-density oscillation occurring at the interface between a metal, such as gold or silver, and a dielectric[4]. For surface plasmon resonance to occur, a p-polarized laser beam is reflected on the sample through a coupling prism at an angle superior to the angle of total internal reflection (figure1a). The reflection at the metal-dielectric interface creates an evanescent field in both the metal and the dielectric[5]. This exponentially-decaying field is strongly attenuated in the metal, but propagates a greater distance (~200 nm) into the dielectric (sensing medium). Optimal coupling between light and plasmons occurs at specific conditions of incidence angle, laser wavelength and analyte refractive index. This coupling appears as a sharp dip in the measured laser reflectance as a function of incident angle (figure 1b).

Changes in cellular morphology, observed through microscopy techniques, are often used to evaluate cellular activity following specific activation[6-8]. Changes such as cell contraction and spreading are the consequences of cellular activation. Recently, SPR has been applied to real-time monitoring of living cells to probe molecular processes in the basal part of the cells (figure 1c), which lies within the evanescent field of the surface plasmon[9, 10].

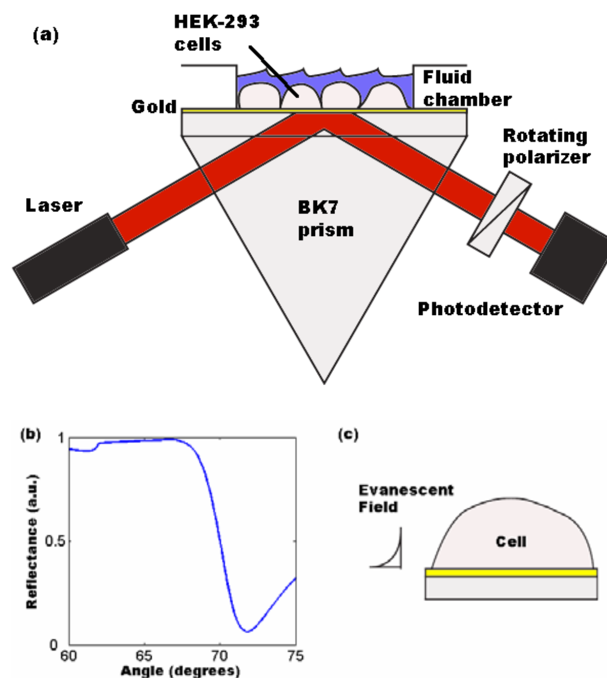

Figure 1: (a) Schematic representation of the surface plasmon resonance apparatus. A laser is incident on the gold-coated sample and the reflectance is monitored by a photodetector. A rotating polarizer allows the use of the s-polarization, unaffected by the surface plasmon, for normalization. (b) Typical SPR angular scan (c) The evanescent field created by the surface plasmon resonance only allows monitoring of events occurring at the basal level of the cell (200nm).

Here, we demonstrate that surface plasmon resonance can be used as a biosensor combined with living cells to evaluate cellular activity following specific activation. To do so, we use three different molecular stimuli for the cells: thrombin as a specific agonist, lipopolysaccharides (LPS) from Escherichia coli as an endotoxin and sodium azide as an apoptotic agent. Then, the surface plasmon resonance response is validated with phase contrast microscopy.

MATERIALS AND METHODS

Cell culture

Human embryonic kidney-293 (HEK-293) cells (Qbiogene Carlsbad, CA, USA, QBI-HEK-293A cells) were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2mM L-glutamine, 2.5µg/ml amphotericin B, 50IU/ml penicillin and 50µg/ml streptomycin (Wisent, St-Bruno, Qc, Canada) at 37ºC in 5% CO_2 incubator. Prior to the experiments, the cells were plated on a SPR glass-gold surface in a 60 mm petri dish and left to grow to confluence. HEK-293 cells were respectively stimulated with 2 units/ml thrombin, 500µg/ml lipopolysaccharides from *Escherichia coli* 017:B8 and 100mM sodium azide (Sigma-Aldrich, Oakville, Ontario, Canada).

Surface plasmon resonance substrates

Standard glass microscope slides were used as base substrates. Prior to metal deposition, glass slides were cleaned in piranha solution to remove any contaminants; afterwards they were placed under vacuum for metal deposition (BOC Edwards evaporator, model: AUTO 306). A chromium adhesion layer (3 nm) and a gold layer (48 nm) were deposited subsequently without breaking vacuum between evaporation. Prior to cell culture, gold surfaces were coated with poly-L-lysine (Sigma, Oakville, ON) to assure good cellular adhesion.

Surface plasmon resonance apparatus

The analysis was performed at room temperature (22ºC) on a custom-built surface plasmon resonance apparatus. The substrate is placed on top of a BK7 coupling prism (Melles Griot, USA) with a layer of refractive index matching fluid (Cargille Laboratories, New Jersey). A 4 mW laser diode centered at a wavelength of 635 nm (Thorlabs, Inc., USA) is reflected off the gold surface and passed through a motorized linear polarizer. As only the p-polarization is coupled into the surface plasmons, the s-polarization is used as a reference to remove the dependence on laser power drift and other time-dependant noise sources. The intensity of the polarized laser beam is measured by a biased photodetector (Thorlabs, Inc., USA) and the signal is acquired and treated in a custom-developed LabVIEW control interface program. Reflectance as a function of angle was measured before each experiment. A fixed angle was then selected in the quasi-linear region of the SPR spectrum and reflectance as a function of time was monitored. Data were treated to remove bulk refractive index changes following injection.

Phase contrast microscopy

The cell morphology changes were recorded using an inverted fluorescence microscope (AxioVert 200, Carl Zeiss, Germany) equipped with a phase-contrast system. All images were captured with a 40X phase-contrast objective, a high sensitivity camera (AxioCam MRm, Carl Zeiss, Germany) and analyzed with AxioVision LE software.

RESULTS

Monitoring laser reflectance over angle

Figure 2 shows two typical SPR angular scans of a confluent HEK-293 cell monolayer, taken before (blue) and after (red) sodium azide stimulation. Phase contrast images of cells illustrate the decrease in surface area caused by the formation of greater intercellular space (arrows indicate correspondence before points at the start of the experiment and 30 min after sodium azide addition). The decrease in the area occupied by the cell on the gold surface causes a decrease in the effective refractive index in the evanescent field, reducing the angle where resonance occurs (blue vs. red scans in the graph). This variation in angle of resonance is thus usable for monitoring of cellular morphology changes.

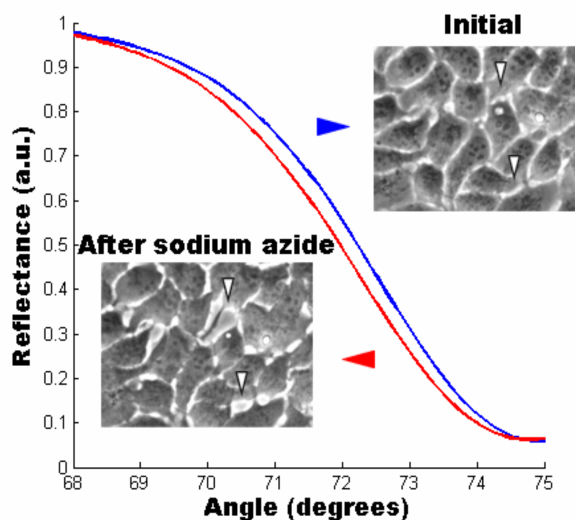

Figure 2: Angular scans of a HEK-293 cell monolayer before (blue) and after 30 minutes (red) of the injection of sodium azide 100mM. Phase contrast microscopy images show the formation of intercellular space as pointed by the arrows 30 minutes after injection (left) compared with the initial conditions (right).

Monitoring laser reflectance over time

Thrombin is a well documented agonist known to cause cellular contraction by the activation of protease

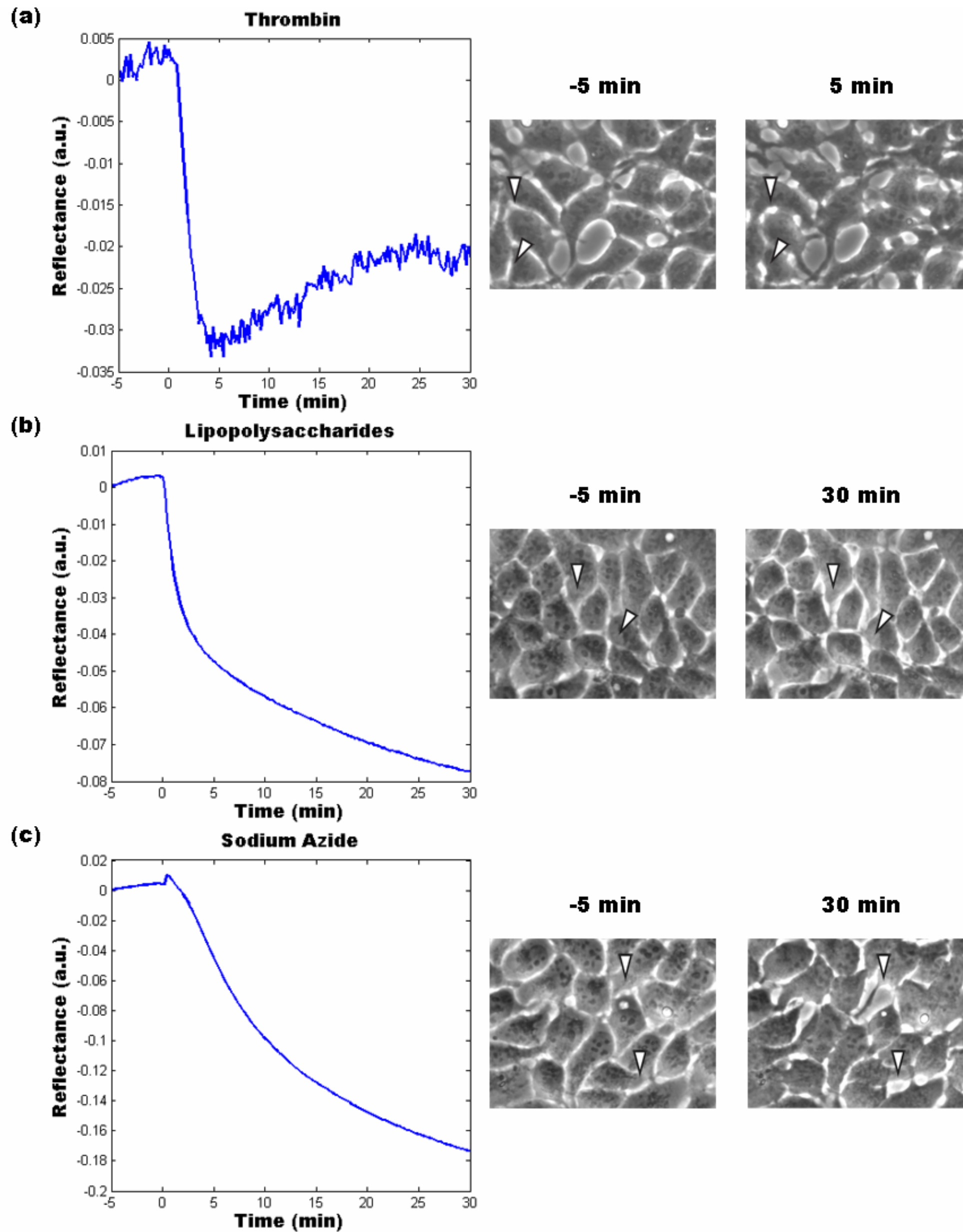

Figure 3: (Left) Laser reflectance as a function of time of a HEK-293 cell monolayer after stimulation by (a) thrombin 2 units/ml, (b) lipopolysaccharides 500 µg/ml from *Escheria coli* and (c) sodium azide 100mM. (Right) Phase contrast microscopy images taken (a) 5 minutes before and 5 minutes after the injection of thrombin. (b) and (c) Phase contrast microscopy images taken 5 minutes before and 30 minutes after the injection of lipopolysaccharides and sodium azide, respectively. Arrows indicate the main morphological changes induced by the selected agent.

activated receptors[11]. The monitoring of SPR reflectance variations after injection of 2 units/ml of thrombin can be seen in figure 3a. We noticed a maximal decrease, equivalent to -0.035 in measured laser reflectance (arbitrary units), immediately after stimulation, which was then followed by a near constant plateau. Phase contrast images, in figure 3a, showed a small cellular contraction, which was in accordance with the measured decrease in SPR reflectance.

Lipopolysaccharides are endotoxins known to affect living cells by promoting pro-inflammatory cellular response by the activation of a receptor complex[12]. The response is often associated with cell rounding[13]. SPR measurements for LPS stimulation are shown on figure 3b. We noticed a rapid and continuous decrease of the measured laser reflectance over the whole duration of the experiment, for a total variation of -0.0807 in reflectance after 30 minutes. Here again, phase

contrast microscopy images clearly showed a corresponding morphology change of the cells.

Sodium azide is a toxin known to cause cell rounding associated with death by inhibiting cellular respiration[14]. Figure 3c shows real-time monitoring of the sodium azide effect on cellular activity. We observed a rapid and strong decrease of the measured laser reflectance distributed over the whole 30-minutes period, equivalent to -0.1781 reflectance units. Phase contrast microscopy clearly showed the formation of large intercellular spaces as a result of cellular contraction.

DISCUSSION

Validation of the SPR response with phase contrast microscopy clearly demonstrates that combining surface plasmon resonance with a confluent living cell monolayer can be used to form a broad-range biosensor that allows the monitoring of cellular activity. This novel biosensor could serve as a rapid detection method to precisely evaluate cellular response in real-time following activation through various agents.

In our study, we have shown that the excitation of cells with different stimuli, such as agonists, endotoxins and apoptotic agents, can be easily translated into a refractive index variation through the monitoring of surface plasmon resonance.

Here, we limited our experiments to the investigation of external agents causing cellular contraction. The possibility to use SPR to observe other kinds of morphological changes remains to be determined. The observed correlation between the SPR signal and cellular morphology suggests that other morphological changes, such as cell spreading and biphasic cell response, will be easily detected with SPR. It is also obvious that control over normal cellular environmental conditions (37 ºC, 5% CO_2) currently limits this biosensor. Indeed, only a few cell types can resist harsh conditions such as ambient temperature and serum-deprivated medium. Furthermore, we pose that a sine qua non condition for SPR monitoring of cellular activity is that it induces morphological changes.

In conclusion, surface plasmon resonance has the capacity to precisely determine cellular activity in real-time. This novel biosensor could potentially be used to conduct high-throughput assays to find new potential pharmacological agents in drug research. It would also find applications in environmental and health care to determine toxin presence and their corresponding cellular response. This biosensor could also be used to study the cell itself in order to better understand complex cellular mechanisms such as endothelial cells cohesion in vascular permeability.

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