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Short communication

Label-free and time-resolved measurements of cell volume changes by surface plasmon resonance (SPR) spectroscopy

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ABSTRACT

Cell volume and its regulation is one of the key players for cellular integrity and a strong indicator for several cell pathologies. But time-resolved volume measurements of adherently grown mammalian cells using established methods, such as extracellular impedance analysis or light microscopy, are complex and time-consuming. In this study, we demonstrate that surface plasmon resonance spectroscopy (SPR) is a powerful transducer device capable of reporting volume changes of cells that are directly grown on the SPR sensor surface. The approach is label-free, non-invasive and provides an outstanding time resolution. In proof-of-principle studies we recorded the volume change of confluent MDCK II cells induced by hypo- or hypertonic stimulation in a time-resolved manner. Comparison of the SPR-based experiments reported here with more recent studies using different approaches suggests a direct correlation between SPR signal shift and cell volume changes.

1. Introduction

Label-free and time-resolved cell volume measurements of viable cells have been a challenging problem in many areas of experimental and diagnostic biomedical science (Kimelberg et al., 1992). Thus, in the past a variety of techniques have been used to tackle this sensoric task. These include electrophysiological methods, like patch clamping (Satoh et al., 1996) and scanning ion conductance microscopy (Korchev et al., 2000), or methods assessing indirectly the mean cell volume by impedance measurements, like Coulter counting (Grinstein and Furuya, 1984) and volume cytometry (Ateya et al., 2005). These techniques are, however, technically demanding, difficult to apply to more than a small number of samples and are highly dependent on a well-defined and constant conductivity of the experimental buffer with and without osmotic loads. Besides the above mentioned electrochemical techniques, sophisticated optical methods, like interferometry (Farinas et al., 1997), light diffraction (Mcmanus et al., 1993), confocal microscopy (Satoh et al., 1996) and various kinds of three-dimensional fluorescence imaging techniques (Allansson et al., 1999; Crowe et al., 1995), have been used for cell volume measurements. Many of these methodologies require complex microscope configurations and/or laborious imaging and computational procedures. Others involve the introduction of fluorescent labels into the cell, which might ultimately impact viability and permit data acquisition only for a limited period of time before photobleaching degrades the signal or phototoxicity becomes apparent. Thus, the development of a simple, efficient and non-invasive procedure, which allows recording the time course of cell volume changes, has clear and extensive application potential.

Here we report on a biosensor based on surface plasmon resonance (SPR) spectroscopy for the detection of cell volume changes. SPR spectroscopy is an optical method for measuring the refractive index of very thin layers of material adsorbed on a metal sensor layer. A fraction of the incident light energy that hits the surface at a sharply defined angle can interact with the delocalized electrons in the metal film (plasmon) thus reducing the reflected light intensity. The angle of incidence and therefore the SPR signal depends on the refractive index of the adsorbed material (Homola, 2008). In the last years it has been shown that SPR sensors in combination with adherent eukaryotic cells as biorecognition elements can be used to record and investigate cellular processes and morphology changes (Robelek, 2009). As volume changes of cells grown on such an SPR sensor surface always go along with multiple cellular processes that induce a change of the refractive index of the cytoplasm reaching into the evanescent measurement field, SPR should be a very helpful tool for the investigation of cell volume changes.

2. Materials and methods

2.1. Solutions

Experimental buffers were based on Dulbecco's phosphate buffered saline solution including 0.5 mM $MgCl_2 \cdot 6H_2O$ and 1 mM

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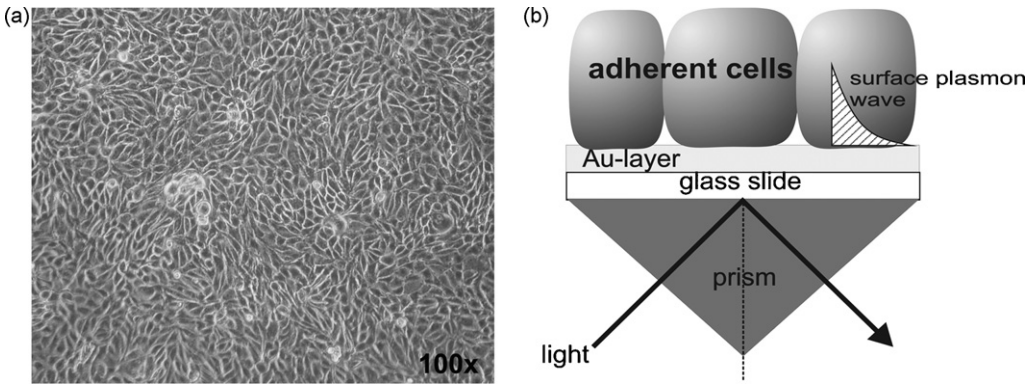

Fig. 1. (a) Phase contrast micrograph of a confluent MDCK II cell layer grown directly on top of a SPR sensor surface. (b) Scheme of the Kretschmann-configured SPR sensor unit for the measurement of cell volume changes.

$CaCl_2 \cdot 2H_2O$ (isotonic running buffer, PBS^{2+}). To increase the osmolarity of this buffer 100, 200 and 400 mM sucrose (Serva) was added, respectively. Osmolarity was checked using an Osmomat 030 Osmometer (Gonotec, Germany).

2.2. Cell culture

Madin-Darby Canine Kidney Strain II (MDCK II) cells were kindly provided by the Institute of Biochemistry, University of Muenster. For stock cultures, the cells were grown to confluence in standard cell culture bottles (25 cm^2, Corning) using Minimal Essential Medium (MEM, 1×) with 1 g/L D-glucose plus 5% (v/v) fetal calf serum, 4 mM L-glutamine and 100 μg/mL penicillin/streptomycin. The cultures were kept in an ordinary humidified cell incubator at 37 °C and 5% CO_2.

2.3. SPR sensor preparation

50 nm gold layers thermally evaporated onto high refractive index glass slides were purchased from MiviTec (Germany). The SPR sensor slides were put in small cell culture dishes and were pre-incubated with 3 ml of the standard cell culture medium (see above) for 5 min. The MDCK II cells were removed from the bottom of the cell culture bottles by standard trypsination and suspended in 2.5 ml culture medium. The suspension was diluted 10-fold with culture medium and added to the sensor containing culture dishes. The sensor/cell system was incubated for another 24 h at 37 °C and 5% CO_2.

2.4. SPR measurement

SPR measurements were performed with a Biosuplar 6 SPR system (MiviTec, Germany) at room temperature. Data was recorded using the manufacturer's software and exported to the software Origin6 (OriginLab, USA) for further analysis.

3. Results and discussion

3.1. Setup of SPR sensor

To realize the SPR sensor concept for cell volume measurements we cultured MDCK II cells directly on top of gold-coated SPR chips (thickness of gold film 50 nm), which were pre-incubated for 5 min with serum containing culture medium. After obtaining a confluent cell layer, the cells were analyzed by phase contrast microscopy. As can be seen from Fig. 1a the adherent MDCK II layers show the typical cobblestone-like morphology of epithelial cells growing on a standard culture substrate. Staining of the cells with trypan blue revealed that the cell membranes are intact and impermeable for this vital stain, indicating cell vitality (data not shown).

After removing the culture medium and washing the cell layers with PBS^{2+}, the sensor surfaces were mounted on top of a glass prism using immersion oil matching the refractive index of the glass slide and the prism. The resulting Kretschmann-configured SPR sensor (Fig. 1b) was covered with a polydimethylsiloxane (PDMS) sealed, 2-channel flow cell. The SPR measurements that were performed using the described sensor setup always recorded the signal of both of these channels while they were continuously rinsed with a 10 μl/min flow of buffer. On one channel, the so named "measurement channel", the SPR signal of those MDCK II cells was recorded, that were stimulated to change their cell volume by changing the osmolarity of the measurement buffer, while the "reference channel" showed the signal of MDCK II cells under constant flow of isotonic experimental buffer. By subtracting the reference signal from the SPR signal of the measurement channel the influence of temperature and pressure drifts was eliminated.

3.2. SPR measurement of cell volume changes

After obtaining a stable SPR signal from both channels, we changed the buffer of the "measurement channel" from isotonic (270 mOsm/kg) to hypertonic (340–553 mOsm/kg) by adding 100, 200 and 400 mM sucrose to the buffer, respectively. This disaccharide is considered as membrane-impermeable so that its presence in the bathing fluid causes a constant hyperosmotic stimulation of the cells. As shown in one of our previous studies using confocal laser scanning microscopy (Steltenkamp et al., 2006) the cells decrease their volume in response to this stimulus due to osmotic waterflow out of the cells (see micrographs in Supplementary Materials). In this study the cellular volume adaptations to these hypertonic stimulations were followed online by the SPR signal. An inverse experiment was performed by applying a hypotonic stimulation, which results in a cell volume increase. Here the isotonic buffer was diluted by the addition of water yielding an osmolarity of 135 mOsmol/kg. For all measurements the "reference channel" was continuously rinsed with the original isotonic buffer. Fig. 2a shows a selection of the reference corrected SPR sensograms that were obtained during the hyper- and hypotonic stimulations of the MDCK II layer. For the hypertonic stimulations we observed a rapid increase of the SPR signal immediately after the cell layer was rinsed with the hypertonic medium. The increase slowed down and reached a new, stable equilibrium level after about 6 min. By changing the osmolarity of the buffer back to the original isotonic value of 270 mOsmol/kg the SPR signal returns to the starting level. We postulate that the SPR signal correlates to the cellular volume change, as the loss of cellular water from the cytosol causes