A surface plasmon resonance imaging system for the stimulated living cell analysis*

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(Received 26 September 2014)

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In this paper, a surface plasmon resonance imaging (SPRI) system for cell analysis is developed for obtaining the surface plasmon resonance (SPR) signal from the interactions between cells and different stimuli. The system is constructed with a red laser light source, a P-polarizer, a glass prism, a 5× objective lens, a charge coupled device (CCD) camera, a gold sensor chip, a polydimethylsiloxane (PDMS) reaction well and a mechanical scanning device. The system is applied to mapping living cells in response to stimuli by characterization of the refractive index (RI) changes. Cell responses to K^+ in KCl solutions with concentrations of 5 mmol/L, 20 mmol/L, 50 mmol/L and 100 mmol/L are collected, which indicates that the SPRI method can distinguish the concentration of the stimuli. Furthermore, cell responses to epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) are studied independently. The binding of EGF receptor (EGFR) and EGF is collected as the first signal, and the internal change in cells is recorded as the second signal. The cell response to VEGF is different from that to EGF, which indicates that the SPRI as a label-free, real-time, fast and quantitative method has a potential to distinguish the cell responses to different stimuli.

Document code: A **Article ID:** 1673-1905(2015)01-0077-4

DOI 10.1007/s11801-015-4171-6

There are many conventional techniques for the evaluation of cell status, such as fluorescence microscopy, flow cytometry and electrical impedance. Most of the cell based assays require tedious labeling methods[1] which are time-consuming and may affect normal cell functions. The development of time-resolved label-free methodologies for the measurement of cellular properties is crucial for the analysis of living cells[2].

Surface plasmon resonance (SPR) is an extremely sensitive optical technique for the study of molecular interactions with advantages of real-time, label-free and kinetics detection at the sensor interface[3-5]. Recently, surface plasmon resonance imaging (SPRI), which is capable of spatially distinguishing refractive index (RI), was applied for real-time imaging of living cell activation[6-8]. Yanase et al[9] detected the RI changes in individual living cells by means of SPRI. Moreover, the SPR response could be used to describe cell growth[10] and evaluate the effectiveness of antitumor drug on tumor cells[11]. When cells were stimulated by extracellular stimuli via receptors, various intracellular events were initiated, which were then detected by SPR quantitatively[12].

In this paper, a homemade SPRI system is developed for cell analyses. SPR signals are obtained from the interactions between cells and different stimuli, such as K^+, epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). This method provides a label-free, real-time, fast and quantitative way to detect RI changes in living cells induced by stimuli.

The homemade SPRI system for living cell analyses is based on the prism coupling mode of the Kretschmann structure. As shown in Fig.1, the proposed SPRI system consists of a red laser light source with peak wavelength of 650 nm, a P-polarizer, a glass prism (ZF4) with an equilateral triangle shape and RI=1.72, a gold sensor chip (50 nm-thick film) with a polydimethylsiloxane (PDMS) reaction well, a 5× objective lens and a charge coupled device (CCD) camera (Basler, A102f). The laser installed on a rotating arm emits a P-polarized parallel light beam which passes through the prism and strikes the sensor surface, and the reflected beam is collected by the CCD detector mounted on another rotating arm. The incident angle can be swept from 40° to 70°. The light intensity in the area of interest from the CCD camera is recorded to

* This work has been supported by the National Basic Research Program of China (Nos.2011CB933202 and 2014CB744600), the National High Technology Research and Development Program of China (No.2014AA022303), and the National Natural Science Foundation of China (Nos.61201079, 61372055, 81371711 and 31100820).

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get SPR signal and images at an SPR coupling angle. The coupling angle is sensitive to the RI changes of medium adjacent to the metal layer supporting surface plasmons. Therefore, the RI changes at the sensor surface induced by the interactions between target analyte molecules and living cells cultured on the sensor surface can be observed as the changes of resonant angle.

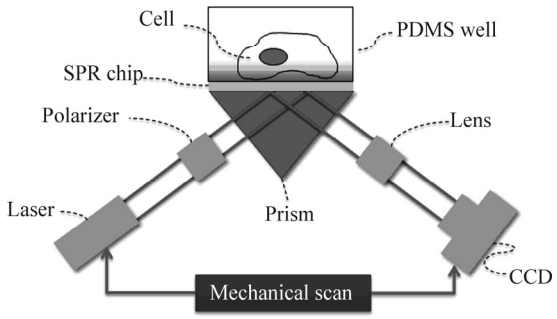


Fig.1 Schematic diagram of the SPRI system for cell analysis

SPRI gold chips were made by sputtering 2 nm-thick chromium and 50 nm-thick gold on 20 mm×20 mm×0.3 mm ZF4 glass slides. A PDMS reaction well was immobilized on top of the glass chips for various biochemical reactions of cells. The PDMS reaction well was made by a mixture of the PDMS oligomer and the crosslinking agent (sylgard 184) with ratio of 10:1. The mixture was degassed under vacuum, poured into a silanized glass mould, and then cured in an oven at 80 ºC overnight. After the glass mould was peeled off, the PDMS reaction well with a square sample reaction hole was formed with internal length of 8 mm, external length of 16 mm and height of 8 mm.

Human hepatocelluar carcinoma cells (HCC-LM3) were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C with 5% CO_2 and 70% relative humidity. On the day before experiments, cells were dissociated with 0.25% trypsin and 0.02% ethylene diamine tetraacetic acid (EDTA) in phosphate buffer saline (PBS) solution. 30–50 HCC-LM3 cells per 1 mm^2 were cultured on the surface of the gold chip for 12 h. Then the cells were maintained in DMEM without fetal calf serum for SPRI analysis.

The incident angle was scanned from 50° to 57°, and an SPR absorption peak of the culture medium was collected at 52.7° as shown in Fig.2(a). SPR image was collected at a fixed angle of 52° for the RI difference between the cells and the culture medium as shown in Fig.2(b), where the bright spots are cells, and the dark areas are the medium.

In order to reduce the interference factors of various proteins in the culture medium, PBS solution was chosen to replace the culture medium in the following experiments. When the signal was stable, the medium was continuously changed by KCl solutions with concentrations of 5 mmol/L, 20 mmol/L, 50 mmol/L and 100 mmol/L to test the SPR response of cells to different concentrations of K^+. The experiments were repeated for 3 times with new chips each time.

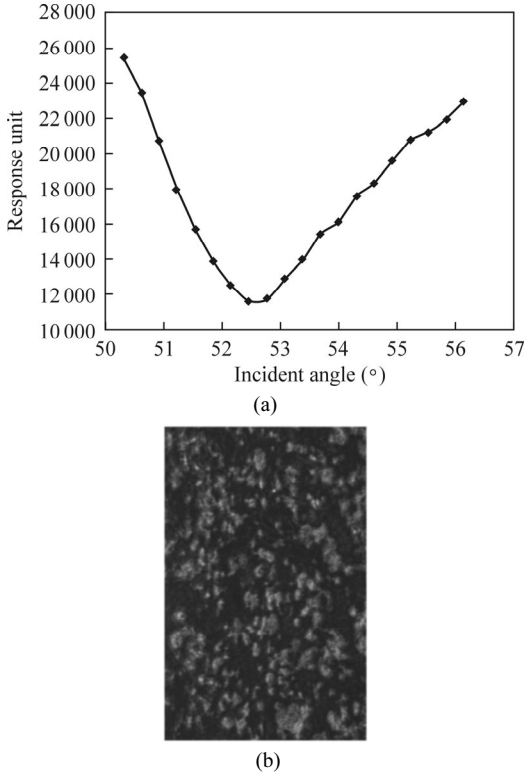


Fig.2 (a) SPR absorption with angle scanning range from 50° to 57°; (b) SPR image of the cultured liver tumor cells

Using two separate cell chips, when the SPR signal was stable in PBS solution, EGF and VEGF were added to stimulate the cells independently. Furthermore, the concentration of VEGF was increased to test the changes of SPR signal. Finally, the PBS solution was injected.

Fig.3 shows the cell responses to K^+ in KCl solutions with concentrations of 5 mmol/L, 20 mmol/L, 50 mmol/L and 100 mmol/L. After KCl was injected, the SPR response units (RUs) of cell areas (series 1–7) increased, while RUs of the reference areas (series 8 and 9) without cells did not change. SPR images of 9 areas for cells and references are shown in Fig.4. The experimental result is consistent with the observation reported by Fontainhas and Shinohara[13,14], where the K^+ stimulation induced the protein kinase C (PKC) translocation to cell membranes in liver tumor cells. From Fig.3, it can be derived that the RU of the stimulated cells is increased with the increase of the K^+ concentration, leading to more protein translocations from the cytoplasm to the cell membrane. Therefore, SPRI method can be used to detect the concentration of the stimuli which can be applied to living cells.

The responses of liver tumor cells to 50 IU/mL EGF are shown in Fig.5. The SPR signal of the cells is initially increased by about 1 500 RU, and then is decreased much

lower than the base line (PBS). Meanwhile, the SPR signals of the reference areas without cells have almost no change. The RU peaks appear at about 100 s after the EGF is injected, and then the signals begin to decrease. More than 80% areas covered by cells have a similar response to the EGF stimulation. The experimental result indicates that EGF can bind with EGFR on living liver tumor cells. In the past 30 years, the studies of EGF and EGFR have revealed the molecular mechanism of EGF reactions with cells[15]. When EGF specifically binds with EGFR existing on cells membrane, it stimulates the tyrosine kinase activity in EGFR complex, which begins to present autophosphorylate and forms the signal transmission in cells. Therefore, cell response to stimuli can be detected by the SPR method dynamically since SPR is extremely sensitive to the sensor surface changes caused by the molecular interactions. In our experiments, it is inferred that the increasing signal during the first 100 s after injecting EGF (the initial signal) is induced by the binding events between the EGF and EGFRs on HCC-LM3 cells. The declining period after 100 s (the second signal) is caused by the internal event taking place in the cells. Liu[10] studied the fixed cell response to EGF by SPR method, and the SPR signal did not decline after EGF bonding to EGFR. Therefore, the second signal detected with SPR can be used as a criterion to evaluate the characterization of cell internal signal transduction process.

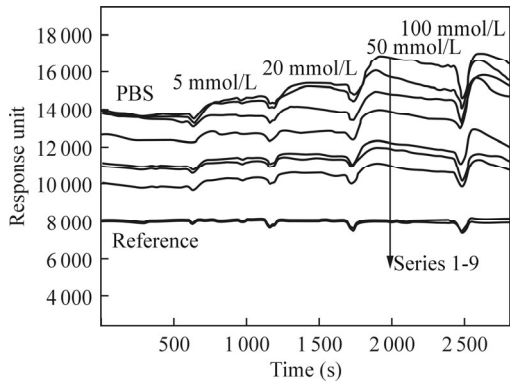
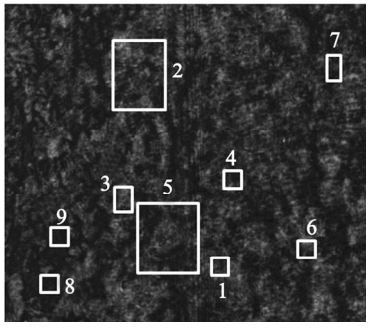
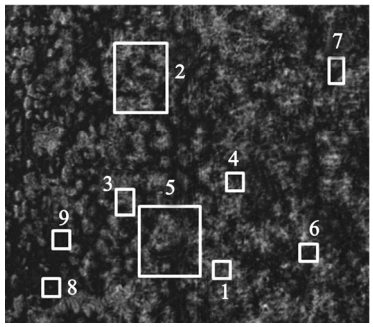


Fig.3 The liver tumor cell responses to KCl solutions with different concentrations of 5 mmol/L, 20 mmol/L, 50 mmol/L and 100 mmol/L in 9 selected areas



(a)



(b)

Fig.4 SPR images of 9 areas of cells (1-7) and references (8, 9) (a) before and (b) after injecting KCl

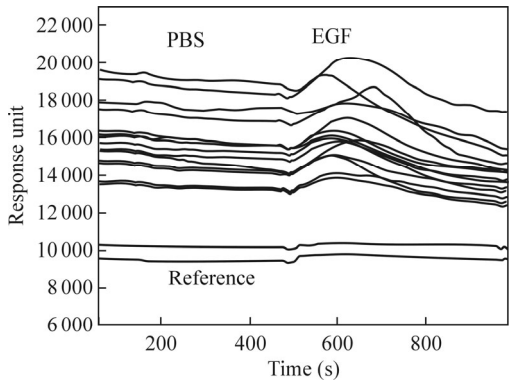


Fig.5 The responses of liver tumor cells to 50 IU/mL EGF

To compare the cell responses to different chemical stimuli, VEGF was injected to the living HCC-LM3 cells, and the process was monitored by SPRI system. VEGF is highly expressed in many tumor cells, and also plays an important role in the occurrence and development of hepatocellular carcinoma[16]. Different from that to EGF, the cell response to VEGF remains stable at a concentration of 150 ng/mL as shown in Fig.6. There is a tiny declining SPR signal as the concentration of VEGF is increased to 1 500 ng/mL. The cell response to VEGF is different from that to EGF, which suggests that the developed SPRI system has a potential to the selective detection of cellular responses to different stimuli.

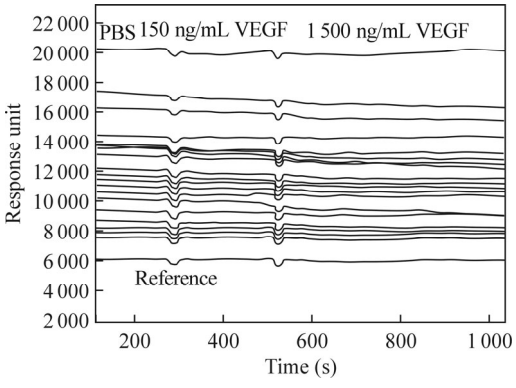


Fig.6 The responses of liver tumor cells to VEGF with concentrations of 150 ng/mL and 1 500 ng/mL

Usually, SPR methods are used to study the affinity interactions of protein molecules, such as the interactions beween antigens and antibodies and beween ligands and receptors[17,18]. In this paper, we make a real-time analysis of living cell responses to stimuli by a homemade SPRI instrument. SPRI, as a fast, label-free, real-time and quantitative method, is good at monitoring the RI in the evanescent field layer (~200 nm), so the cell membrane changes and the signal transduction can be studied.

In this paper, an SPRI system is constructed with a red laser light source, a P-polarizer, a glass prism, a 5× objective lens, a CCD camera, a gold sensor chip, a PDMS reaction well and a computer controlled mechanical scanning device. The SPR images and signals are collected by the CCD camera and displayed on a homemade software. The SPRI system is used to study the RI changes on surfaces containing living cells subjected to stimuli, especially in the region near the membrane. Cell responses to K^+ with different concentrations are inferred to be correlated to the PKC translocation to cell membranes in liver tumor cells. Comparing the cell responses to two kinds of growth factors like EGF and VEGF, we can get the binding signal by the initial period detection and the cell internal transduction signal by the second period signal for EGF stimulation. The SPR response can be used to describe the cell responses to various stimuli as well as in many other areas.

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