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A spectral imaging array biosensor and its application in detection of leukemia cell

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ABSTRACT

A new type of array immunosensor was developed by combining surface plasmon resonance (SPR) and spectral imaging techniques. The system consisted of a monochromator as the wavelength scanning light source, a polarizer, Kretschmann–Raaether attenuated total reflection (ATR) configuration including array sensor chip, and a CCD camera. The images of transmitting light from ATR were recorded versus the wavelength. By averaging gray scales of the pixels in the area of every gold spot from the image series, the complete spectral resonance curve of all sensing spots on the array can be extracted in parallel. The performance of the developed system was evaluated by analyzing interactions of the anti-CD33 monoclonal antibody to its target leukemic cells using 11 cases of human bone marrow specimens. The specimens were also analyzed with flow cytometry method (FCM) for comparison. The initial results measured by the immunosensor array were corresponded with that of FCM, indicating that the developed parallel method might be clinically suitable for immunophenotyping of acute leukemias. The new sensor array system showed the merits of high-throughput, high sensitivity, high specificity, label free and operation convenient. Spots numbers of the array could be increased if suitable technology were adopted for manipulating the micro bio-liquids on the sensor array chip.

1. Introduction

The surface plasmon resonance (SPR) biosensor is sensitive to the changes of the refractive index of biomaterials at the interface between a thin Au film and an ambient medium and thus it is able to characterize biomolecular interactions on the surface in real time without labeling [1]. Since the appearance of SPR biosensor, remarkable progress has been made in the development of SPR sensor techniques and their applications in many fields such as environmental monitoring, biotechnology, medical diagnostics, drug screening, food safety, and security [2–4]. However, the majority of existing SPR sensing devices offer only mono-channel and thus their throughput is rather limited. Therefore, one of the main challenges for SPR sensor research is to explore high-throughput capability [5–7].

The most straightforward approach to multichannel SPR sensing is SPR imaging technique [8]. In a SPR imaging setup, the incident angle is usually fixed adjacent to the resonant angle and the measured parameter is the intensity variation of the light reflected from the SPR sensing surface using a CCD camera. The distribution of intensity could be used to detect variations of refractive index on the sensor surface [9,10]. Such a SPR imaging technique had been exploited to study protein binding to a DNA array [7], surface analysis of C-reactive protein binding [11] and to detect local electrochemical processes on patterned surfaces [12]. However, it provides lower resolution than classical SPR systems because it measures the intensity variation measurement at a single position of the resonance spectrum, instead of measuring a complete surface plasmon resonance curve [13]. In this paper, we present an array biosensor system based on spectral SPR imaging which aims at keeping the high resolution of mono-channel SPR sensor based on the full resonance curve measurement while introducing an array chip to perform multi-channel measurement.

Using the developed system, the leukemia cells from human bone marrow were detected by measuring the expression of CD33, a type of myeloid-specific antigen. The CD33 antigen is a 67-kD transmembrane cell-surface glycoprotein expressed on both mature and immature myeloid cells, and on erythroid, megakaryocytic, and multipotent progenitors. The CD33 antigen has served as a cell surface marker of acute myeloid leukemia (AML) for clinical diagnosis of leukemia and therapeutic targeting in AML. The analysis of CD33 and other surface markers have been performed generally with flow cytometry (FCM) that takes advantage of the affinity bind-

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ing of antibodies specific for cell surface antigens [14]. However, there are still limitations in the FCM analysis primarily associated with low throughput of analysis and difficulty in optimizing analytical conditions [15–17], as well as the high cost of instruments and antibody fluoresce labeling. These negative aspects will be critical when lots of surface antigens are screened in a high throughput manner. Some of the above-mentioned difficulties associated with FCM may be overcome by antibody arrays. Recently, many attempts have been made on the development of antibody arrays that are designed to investigate the interactions of many antibodies with their specific ligands in one experiment operation [18–21]. Antibody arrays had been also used for profiling surface antigens expressed on living cells, including prostate cancer cells [22], leukemia cells [23–26], and neural stem cells [27,28]. However, all above antibody arrays need labeling. In this study, using the developed array sensor system, leukemia cells of AML were probed by affinity binding of cells to the specific antibody spots on an array without labeling. The experiment results were compared with FCM.

2. Materials and methods

2.1. Materials

3-Mercaptopropionic acid (MPA, 99%) and *N*-hydroxysuccinimide (NHS) were purchased from Alfa Aesar, USA. *N*-Ethyl-*N*¢-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC HCl, 99%) was purchased from Shanghai Medpep Co., Ltd. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich Chemical Company (St. Louis, USA). Ethanolamine and other chemicals were purchased from standard commercial sources and were of analytical grade.

Anti-CD33 mAb were purchased from Beckman Coulter Inc. The human bone marrow specimens were collected from the clinically suspected AML patients in the First Affiliated Hospital of Medical College of Xi'an Jiaotong University.

2.2. Preparation of biochip

SPR sensor chips were prepared as follows: BK7 glass slides were sonicated in soapy water, distilled water, acetone and ethanol, respectively, for 10 min in an ultrasonic bath. Then, the slides were sufficiently rinsed with distilled water and dried with nitrogen stream and were coated with chromium thin adhesion layer. The 3×3 gold spot arrays (diameter of the spot: 1.0 mm) were fabricated by depositing Au films (thickness: 50 nm) on glass slides using a sputtering apparatus. The fresh gold-coated array slide was immersed in 0.1% MPA solution for 12 h to form a self-assembled monolayer (SAM) on the surface of gold spots. After the surface self-assembly process, the array chip was washed in methanol and water, respectively. Then, the carboxyl groups on the surface were activated by incubation with a solution of 50 mM *N*-hydroxysuccinimide and 200 mM *N*-ethyl-*N*-(ethylaminopropyl)-carbodiimide hydrochloride in distilled water for 30 min. A polydimethylsiloxane (PDMS) holey cell was masked on the sensor chip array to confine the target solutions on the gold spots. Anti-CD33 mAb solutions were dropped into the PDMS cell holes on the chip for immobilizing anti-CD33 mAb on the spots of array chip. After incubating for 1 h at 37 °C, the gold spots were rinsed, respectively, with PBS (pH 7.4) twice and then incubated in 1.0 M ethanolamine–HCl (pH 8.5) solution for 30 min at 25 °C to block the residual carboxyl groups. Consequently the surface was rinsed with PBS and deionized water to remove the unattached ethanolamine. The above prepared chips were stored for standby at 4 °C.

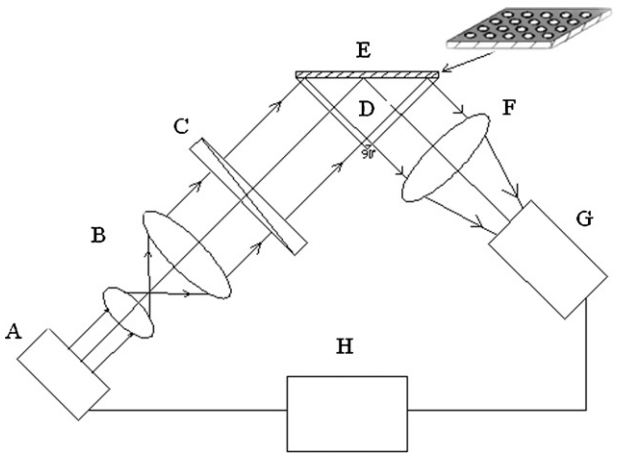

Fig. 1. Schematic of the spectral SPR imaging system. (A) Monochromator, (B) collimator, (C) polarized, (D) prism, (E) sensor chip, (F) lens, (G) CCD camera, and (H) computer.

2.3. Establishment of spectral SPR array biosensor

An array biosensor system based on spectral SPR imaging was developed with the Kretschmann configuration. The schematic of the system is shown in Fig. 1.

A monochromator with the wavelength ranging from 440 nm to 950 nm and a wavelength resolution of 0.8 nm was used as the light source and a polarizer was positioned at the input light path to obtain p-polarized light. The collimated beam was guided towards the prism with a suitable incident angle. Index-matching fluid was used to achieve optical contact between the BK7 prism and the array sensor chip. The reflected light from the array sensor chip were collected into a CCD camera with imaging optics. The camera and monochromator were controlled by a computer. Software programmed using Matlab language was developed to control the monochromator wavelength scanning, image acquiring and saving. The gray scales of the images were recorded responding to the scanning wavelength over 440–950 nm region. The SPR spectral curve of every gold spot was extracted in parallel from the local gray scales of the spot in image series. It exhibits a minimum in the reflection spectrum of SPR resonance. We use the wavelength shift as a quantitative measurement of the changes in refractive index of the sensor surface. When AML cells contact with the surface of sensor chip, the surface antigens CD33 would specifically bind to the spots of the array sensor chip and thus it would result in the shift of corresponding resonance wavelength.

2.4. Flow cytometry analysis

For comparison, flow cytometry analysis (FCM) was carried out. The analysis procedure of FCM was described elsewhere in detail [14]. Briefly, the bone marrows were treated with a lysing solution to remove red blood cells while preserving the leukocytes which were then isolated by centrifugation. The isolated cells were washed twice in PBS, re-suspended in PBS to a density of 1×10^6 cells/mL, and incubated with anti-CD33-PE monoclonal antibody at room temperature in dark for 30 min, while mouse anti-human IgG1 was used as the homotype control. Then the cells were washed by PBS twice, fixed by 1% formaldehydum polymerisatum, and analyzed on a Partec CyFlow space cytometer, running FloMax software.

3. Results and discussion

The leukemia cells in the marrow aspirates of AML patients were analyzed by the established spectral SPR imaging system using the