Sensors & Actuators: B. Chemical 279 (2019) 267–273

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

High-sensitivity glycated hemoglobin (HbA1c) aptasensor in rapid-prototyping surface plasmon resonance

Chen Guang Zhang[a], Shwu Jen Chang[b], Kalpana Settu[c], Ching Jung Chen[d,*], Jen Tsai Liu[e,*]

[a] School of Electronic, Electrical and Communication Engineering, University of Chinese Academy of Sciences, PR China
[b] Department of Biomedical Engineering, I-Shou University, Kaohsiung City, 82445, Taiwan
[c] Department of Electrical Engineering, National Taipei University, Sanxia, Taiwan
[d] School of Opto-Electronic Technology, University of Chinese Academy of Sciences, PR China
[e] College of Materials Science and Opto-Electronic Technology, University of Chinese Academy of Sciences, PR China

ARTICLE INFO

Keywords:
3D printing
Surface plasmon resonance
Aptamer
HbA1c
Kinetics parameters

ABSTRACT

Diabetes mellitus is one of the most common noncommunicable diseases in the world. Fasting blood glucose level is one of the diabetes diagnostic criteria, but it is readily affected by stress, exercise and many other factors. Glycated hemoglobin (HbA1c) is an important clinical index for diabetes and related diseases. In this study, a cheaper, rapid-prototyping, high-sensitivity angle-scanning surface plasmon resonance (SPR) was developed by fused deposition modeling (FDM) 3D printing technology for HbA1c detection. The sensor chip is based on an aptamer that has high affinity and high specificity to HbA1c. The results showed that this HbA1c-specific aptamer had high specificity for HbA1c, and the calculated K_D was 6.13×10^{-8} M. The linear detection response of HbA1c appeared in the range of 18–147 nM, with a detection limit of 1 nM. This SPR HbA1c detection system could be a promising platform for developing clinical point-of-care diagnostic applications.

1. Introduction

Diabetes mellitus is one of the most common noncommunicable diseases in the world. Statistics in 2013 showed that there were approximately 382 million people suffering from diabetes, and the number of diabetes cases is increasing fast and is predicted to reach 592 million by 2035 [1]. Elevated glucose during fasting is one of the widely used diabetes diagnostic criteria, but it is readily affected by stress, exercise and many other factors. Compared with glucose level, the amount of glycated hemoglobin (HbA1c) reflects the average concentration of glucose over the past 100 to 200 days [2]. Glycated hemoglobin (HbA1c) is a glycated protein formed by a nonenzymatic reaction of glucose in the human body [3]. The HbA1c level, defined as the ratio between HbA1c concentration and total hemoglobin concentration, is an important clinical index for diabetes and related diseases [4]. Currently, HbA1c is measured by fluorescence sensing, cation-exchange chromatography, electrophoresis, and boronate affinity chromatography in clinics. However, these methods have some limitations, such as the need for labeling assays, drug interference or error due to wide detection ranges [5].

Aptamers are oligonucleotide or peptide molecules that bind to a specific target molecule. Aptamer-based assays hold great promise to replace antibody-based assays, as aptamers are relatively cheap and easy to store, even at room temperature [6]. Li et al. [7] reported that the HbA1c-specific aptamer selected by SELEX can be chemically synthesized easily with high reproducibility at relatively low cost. The HbA1c aptamers showed high specificity and high affinities. Thus, it has great potential to replace the HbA1c antibody for the detection of HbA1c. Aptamer-based sensors (aptasensors) are mainly divided into two categories, electrochemical [8] and optical [9]. Electrochemical aptasensors are widely used due to their high sensitivity and low cost without any optical devices. However, they are usually affected by an unstable structure of aptamer on the electrode. Optical aptasensors are often based on labeling methods such as chemiluminescence or well-developed fluorescence probes. It is an indirect method to detect the target, it is difficult to collect all signals in time, and fluorescence decays gradually with time. Additionally, the aptamer-modified fluorescent probe might influence the DNA structure and affect the binding affinity. However, due to the advantages of DNA aptamers that have high affinity, high specificity and ease of storage, they have been commonly used in the existing biomolecule detection probes. For this reason, it is very important to develop a direct detection method without any labeling.

Surface plasmon resonance (SPR) is a surface-sensitive optical

* Corresponding authors at: No.19A Yuquan Road, Beijing, 100049, PR China.
E-mail addresses: cjchen@ucas.ac.cn (C. Jung Chen), jtliu@ucas.ac.cn (J.T. Liu).

https://doi.org/10.1016/j.snb.2018.09.077
Received 10 February 2018; Received in revised form 11 September 2018; Accepted 18 September 2018
Available online 19 September 2018
0925-4005/

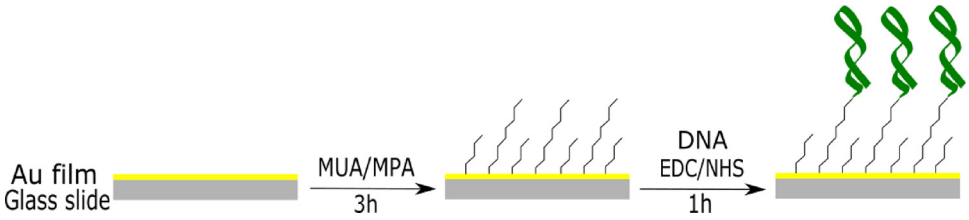

Fig. 1. The scheme of the sensor modification process.

technique that is used to study a thin layer on a metal surface. SPR is a powerful analytical technology that can detect the thickness of the films absorbed onto the sensor surface and interactions between biomolecules, such as antigen-antibody or protein-DNA [10–14]. Compared with traditional detection methods, such as high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assays (ELISA), and fluorescence sensing methods, the major benefits of SPR-based sensors are the potential for label-free detection of analytes and the ability to overcome additive interferences. Moreover, SPR has numerous advantages, such as reliable instrumentation, automation, disposable sensor chips, and versatility due to the wide variety of surface chemistries and assay methods available for various biomolecules. SPR has also been applied in chemical, biology, agriculture, environment and food safety detection [15–21]. However, SPR systems usually require expensive equipment and complicated optics. The cost of commercial SPRs varies from $10,000 to $5,000,00, and the refractive index resolution ranges from 10^{-5} refractive index units (RIU) to 10^{-7} RIU. In previous studies [22–26], many low-cost SPR systems have been introduced, but the resolution and dynamic range are often sacrificed. Hence, developing an efficient SPR system with a modular design and use of advanced hardware resources is of great interest.

3D printing, also called additive manufacturing, has been a tool for developing rapid prototyping products since the 1980s. After the expiration of 3D printing technology patents, this field has witnessed great growth [27]. According to the open-source RepRap project, desktop FDM 3D printers become more popular among users, manufacturers and researchers due to their simplicity, cost-effectiveness and versatility. Application of FDM 3D printing technology is fast emerging in the development of prototypes, scientific tools and medical equipment [28–31]. Using 3D printing technology to design an SPR is an interesting and attractive option. Recently, some studies have demonstrated the use of 3D printing in developing SPR platforms. Hasan et al. [25] utilized the 3D printing technique to print the device holder for a smartphone-based SPR imaging platform. That system has a dynamic range less than 0.02 RIU, which limits the application for measuring large refractive index change. Hence, utilizing 3D printing technology to construct an SPR modularization for developing a high-sensitivity SPR system seems to be challenging.

In this study, a low-cost, rapid-prototyping, high-sensitivity angle-scanning SPR was developed by FDM 3D printing technology for HbA1c detection. To the best of our knowledge, this is the first study to develop an SPR system based on 3D printing SPR modularization for HbA1c detection. The SPR sensor chip was immobilized with an aptamer that had high affinity and high specificity to HbA1c. In addition, using high-sensitivity SPR, we sought to detect HbA1c directly without any labeling and to arrive at a proper diagnosis in a clinical system.

2. Materials and methods

2.1. Chemicals and reagents

DNA sequences were purchased from Beijing Chief-East Tech Co., Ltd. (Beijing, China). The sequence of the HbA1c-specific DNA aptamer used in this work was 5′-GGC AGG AAG ACA AAC ACA TCG TCG CGG CCT TAG GAG GGG CGG ACG GGG GGG GGC GTT GGT CTG TGG TGC TGT-3′. Glycated hemoglobin (68 kDa) was purchased from ProSpec-Tany TechnoGene Ltd. (Ness Ziona, Israel). 11-Mercaptoundecanoic acid (MUA) was purchased from Kuer Co. Ltd. (AnHui, China). *N*-Hydroxysulfosuccinimide sodium salt (sulfo-NHS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 3-mercaptopropionic acid (MPA) were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS) was obtained from Beijing Biotopped Science & Technology Co. Ltd. (Beijing, China). Absolute ethyl alcohol and NaCl were purchased from Beijing Chemical Works (Beijing, China). Deionized water obtained from a CCT-3300 water purification system was used in all the experiments. Matching oil series E(1.520) was purchased from Cargill Dow LLC (USA). Polylactide (PLA) 3D printing filaments were purchased from 3D Systems (USA).

2.2. Preparation of HbA1c aptamer sensor

The gold sensor chip was first washed with SDS solution and exposed under UV-ozone for 20 min. The sensor chip was then immersed in 10 mM 3-mercaptopropionic acid and 11-mercaptoundecanoic acid (volume ratio 10:1) for 3 h. Then, the sensor chip was rinsed with absolute ethyl alcohol. Subsequently, the chip was incubated for 1 h in a solution that contained 1 μM DNA, 5 mM EDC and 3 mM NHS. Finally, the DNA aptamer–immobilized gold sensor chip was rinsed in deionized water and dried with a nitrogen gun. The preparation process of the aptamer sensor is schematically shown in Fig. 1

2.3. SPR system design

The developed rapid-prototyping angle-scanning SPR system block diagram is shown in Fig. 2a, and the realized SPR device is depicted in Fig. 2b. Light from the laser source was first p-polarized by a polarizer, then projected onto the sensor surface via a triangle prism. The reflected light from the sensor surface was collected by a photodiode. The collected raw signal was amplified by a homemade amplifier circuit and sampled by the built-in ADC (analog to digital converter) unit of ATmega2560 microprocessor. The rotating platform could drive the system to scan from 40° to 72°. When performing the measurements, an angle range of 3° around the resonance angle was scanned at a speed of 0.3°/s instead of scanning a whole SPR spectrum. A peristaltic pump was used to transport the sample.

2.4. Electric circuit unit

Fig. 2c shows the block diagram of the electric circuit associated with the proposed SPR system. Two microcontroller units (MCUs) were used to control the system hardware. The rotating platform comprised of the mechanical driving unit and an end-stop sensor. The end-stop sensor was used as the coordinate origin reference. All the above-mentioned components, including the peristaltic pump and ATmega328, were controlled by ATmega2560 via different ports. The amplified signal from the photodiode was sampled at 1 kHz by the ATmega2560 built-in 10 bit Analog-to-Digital Converter (ADC) module. ATmega328 MCU was used to monitor the system temperature. Low temperature drift coefficient negative temperature coefficient (NTC) resistors (103AT-4 shape1) were employed as the temperature sensors. Signals from NTC resistors were quantified by a 16-bit high-precision ADC ADS1115. ATmega328 obtained the temperature information from