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A capacitive surface stress biosensor for CSFV detection

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ABSTRACT

Capacitive biosensors are increasingly popular in biomedical analyses. In order to satisfy the requirements in medical diagnostics, such as high sensitivity and quick response, this paper presents a developed capacitive biosensor based on the surface stress to detect classical swine fever virus (CSFV) antigens. The developed biosensor is composed of a substrate layer and a sensitive layer. The sensitive layer is functioned with antibodies for CSFV antigens. Once the CSFV antigens bind to the antibodies, the increased surface stress will result in convex deformation, converting to capacitance changes. Scanning electron microscope (SEM) and atomic force microscope (AFM) are two methods used to monitor the surface changes. The experimental results demonstrate that the sensing electrode modification process is successful, and the surface stress induced by the binding of antibodies and CSFV antigens can cause variations of capacitance. In conclusion, the developed capacitive biosensor can be used to detect the CSFV antigens, and it has the potential to detect other antigens in the future.

1. Introduction

Biosensors are developed as a cross-integration of emerging technologies from a variety of disciplines of biology, physics, medicine and others. It is one of the hottest areas of biotechnology research. Since 1962, Clark and Lyons had presented the basic biosensor concept and made the first biosensor based on the enzyme electrode to measure blood glucose [1]. Many kinds of biosensors are invented for environmental monitoring, food analysis, DNA analysis and other research fields [2–4].

Surface stress-based biosensors, as an important kind of biosensors, attract lots of attention for their advantages, such as short response time, label free, and typical sensitivity at femtojoule, nanogram level. These biosensors use the underlying concept in any binding reaction without energy change, which in result offers a universal platform for chemical and biological sensing. The sensitive elements of these biosensors, composed of a selective layer and the transducer, are widely studied and developed to improve their performances. In addition, most studies of sensitive elements are focused on micro-cantilevers and micro-membranes. However, the cantilever geometry is not best suitable for sensing in the aqueous media, and can lead to the reduction in the signal-to-noise ratio [5]. Then, micro-membranes are studied as sensitive elements to detect surface stress changes. And silicon [6], silicon oxide [7] based materials are replaced by low stiffness materials, such as Parylene, PMMA and PDMS, to solve the problem that membranes are less compliant than cantilevers [8,9]. In general, optical detection and electrical detection are the two main methods in signal detection. The optical detection has some disadvantages compared to the electrical detection. It can cause thermal effects that are troublesome for sensitive elements and are not portable. However, electrical detection method can overcome these problems. And, capacitive detection method, one kind of electrical detection, has been used to biosensors since 1996 [10]. The sensing technique can be found in many applications for the detection of proteins, DNA, antibodies and antigens [11–14]. Recently, capacitive surface stress biosensor is an attractive research area due to the advantages of high-responsive accuracy and simple operation [15,16].

Classical swine fever, which is caused by CSFV, is one of the virulent diseases in domestic pigs. It is characterized by high spreading speed, severe infectiousness and high mortality. It can cause a great economic loss to pig farming industry. Parallelly to the increasing knowledge of CSFV, some diagnostic methods have been developed at the same time. The isolation of CSFV is "gold standard" to detect CSFV [17]. Although this method is still in application, there are drawbacks of this technology such as long processing time, intensive labor force and complex operation [18]. Moreover, the real-time polymerase chain reaction (PCR), reverse transcriptase-polymerase chain reaction (RT-PCR), and reverse transcription loop-mediated isothermal amplification (RT-LAMP) are used for detection of classical swine fever [19–21]. These CSFV detection methods are based on molecular biological diagnostic tools.

With rapid development, biosensor is becoming a new method for medical diagnostics [22,23]. Hence, a new poly-dimethylsiloxane (PDMS) micro-membrane capacitive surface stress biosensor is used

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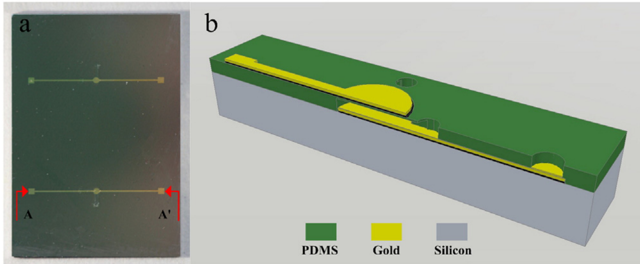

Fig. 1. Capacitive surface stress biosensor. (a) Top view of a real biosensor; (b) The schematic diagram of A–A'cross-section view.

Table 1
The main parameters of the biosensor.

Material	Shape	Radius (μm)	Thickness (μm)
Chromium film of top electrode	Circular	250	0.01
Gold film of top electrode	Circular	250	0.04
Cavity			3
PDMS membrane	Circular	250	1
Chromium film of bottom electrode	Circular	240	0.02
Gold film of bottom electrode	Circular	240	0.04

for the CSFV detection, which is label-free, rapid, high sensitivity and free energy transform. The design and fabrication of the biosensor are presented in our previous work, achieving the integration of PDMS processing with conventional processes [24]. In addition, the bonding technique, which uses uncured PDMS as the intermediate layer, has been proved to be suitable for the binding between the biosensor and the glass microfluidic components. The Finite Element Analysis is used to optimize the sensor geometry parameters. It is found that the circular electrode has smaller fringing effect than the square electrode. Hence, the shape of the proposed biosensor electrodes is designed to be circular. SEM and AFM are used to get information about the biosensor's surface topography and roughness, respectively, verifying the surface changes in the experiment. At the same time, the capacitance measurement method is used to detect the variations of capacitance induced by the CSFV.

2. Capacitive surface stress biosensor

The capacitive surface stress biosensors that are designed and fabricated in this paper are illustrated in Fig. 1a. There are two sensitive structures in one biosensor chip. The one is used as the standard signal for reference. The other one is used to detect CSFV antigens. Therefore, compared with the capacitance values of two sensitive structures, it is easier to tell whether CSFV is detected. The sensitive structure is made up of bottom gold electrode, PDMS membrane and top gold electrode as shown in Fig. 1b. The top gold electrode is placed on the surface of PDMS membrane, which is used to form functional layer to recognize specific targets. The bottom gold electrode is plated on the surface of silicon wafer. These two gold electrodes form a capacitor, and the capacitance can be monitored to reflect the distance changes between the two electrodes. The capacitance can be calculated by the Eq. (1):

$$C = \varepsilon A/d \tag{1}$$

where, ε is the dielectric constant, A is the area of the electrodes and d is the distance between them. The detailed parameters of the biosensor are extremely important to the sensitivity. Herein, the structure of the developed capacitive surface stress biosensor is studied systematically including the characters of size, shape and depth, which reduces the effects of the external environment and improves sensor sensitivity [15]. The main parameters of the biosensor are shown in Table 1. If biological substances attach on the membrane, deflections of the membrane will be produced by the induced surface stress, and translating distance changes to capacitive signal. The detailed principle on deflections of the membrane induced by surface stress is analyzed in our previous research [16].

3. Materials and equipment

Alkanethiol self-assemed monolayers (HS-(CH_2)n-X) are widely studied to construct SAM(self-assemed monolayer). They can be immobilized stably on Au through the S–Au bond and van der Waals force [25], and it is available for their functional groups to bind with analytes. Herein, according to the molecule structure of the CSFV, the HS–(CH_2)$_{10}$–COOH (11-mercaptoundecanonic acid, 1 mM/L) was used to functionalize the biosensor to form a SAM. The biosensor was immersed in 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide /N-hydroxysuccinimide (EDC/NHS, 0.3 mM/L) solution for one hour. Antibodies for swine fever could be dropped on the surface of gold electrode. The indirect haemagglutination assay kit and antibodies for swine fever(diluted in 1:30 proportion) were purchased from Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science. Bovine serum albumin (BSA, 0.1%) was used to block the unreached space, reducing binding chances with nonspecific antibodies. Phosphate-buffered saline (PBS, 0.01 m/L) was used to rinse the excess antibodies.

The capacitances of the biosensor were detected by Agilent B1500A (Agilent Technologies, USA). MIRA3 LMH (Tescan, Czech Republic) was used for SEM images. NX-10 (Park Systems, Korea) was used for AFM images.

4. Results and discussion

4.1. Sensing electrode modification process

The sensing electrode modification process of capacitive surface stress biosensor can be found in Fig. 2. The initial surface of the biosensor was a naked Au membrane. Then the biosensor was immersed in

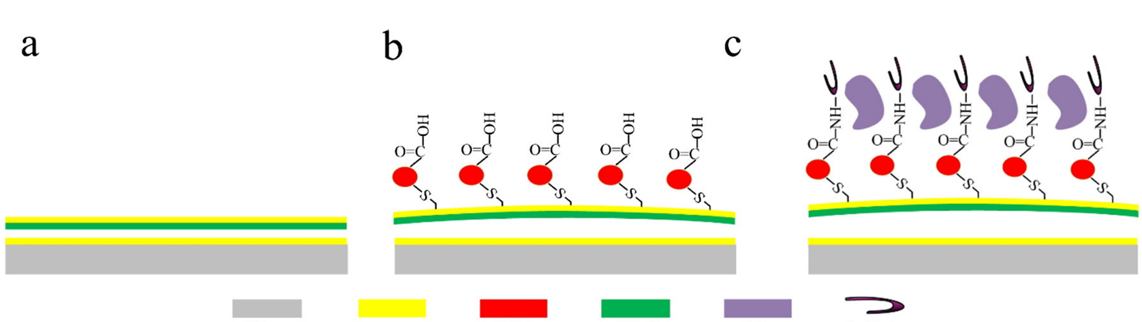

Fig. 2. Schematic diagram of the capacitive surface stress biosensor in modification process.