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Detection system based on magnetoelastic sensor for classical swine fever virus

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ABSTRACT

In this paper, a magnetoelastic (ME) sensing system for the detection of classical swine fever virus (CSFV) is presented. The detection system comprises a test paper and a measurement circuit. The test paper consists mainly of an ME biosensor to detect the CSFV. Based on the impedance analysis technique, the measurement circuit is designed to measure the resonance frequency of the ME biosensor. The anti-CSFV IgG is immobilized onto the ME sensor surface to form the ME biosensor through a physical absorption approach. The experimental results show that the shift in the resonance frequency of the ME biosensor increases with the augmentation of the CSFV concentration. The effectiveness of the combination between the anti-CSFV IgG and CSFV is confirmed by the scanning electron microscopy (SEM) images, the sandwich-based enzyme-linked immunosorbent assay (ELISA) analysis, the interference study and the reference biosensor test method. The resonance frequency shift is linearly proportional to the concentration in the range from 0 to 2.5 μg/ml, and becomes sub-linear at higher concentrations. The ME biosensor for CSFV detection has a sensitivity of about 95 Hz/μg $\cdot$ ml^{-1}, with a detection limit of 0.6 μg/ml.

1. Introduction

Classical swine fever (CSF), caused by the classical swine fever virus (CSFV), is a devastating and highly contagious disease of swine which results in serious financial losses in the world (Edwards et al., 2000). Several countries, including Australia, Canada, New Zealand, the United States of America (USA) and some Member States of the European Union (EU), have successfully eradicated the virus through the implementation of strict control measures. However, in other parts of the world with significant pig production, the CSFV is still present, hindering the development of animal husbandry. Thus, it is included in the Office International des Epizooties (OIE) List A (Moennig, 2000).

During the past years, the CSFV detection has been extensively researched due to the substantial economic damage inflicted by this virus on the pig industry. Various methods have been developed for CSFV detection, such as RT-LAMP (Chen et al., 2010), flow cytometric analysis methods (Gunasekera et al., 2000), reverse transcriptase polymerase chain reaction (RT-PCR) analysis (Liu et al., 2011), colloidal gold-based immunochromatographic assay (Luo et al., 2014), and optical assay (Shen et al., 2007). However, these methods have some limitations, such as the long time required for sample preparation, complicated testing process, expensive instruments and costly maintenance.

Magnetoelastic (ME) sensors made of amorphous, ferromagnetic metallic glass ribbons, for example Metglas 2826MB, have received considerable attention because of their significant advantages of high sensitivity, low cost and wireless sensing. Due to the Joule effect, the ME sensor can vibrate longitudinally at a characteristic resonant frequency under the superimposition of both alternating (AC) and static (DC) magnetic fields (Quandt and Ludwig, 2000). The AC magnetic field is used to impart energy into the ME sensor, causing it to oscillate. The purpose of the DC magnetic field is to select the “operating point” on the magnetization curve of the sensor. As the resonant frequency is easily affected by the surrounding medium, the ME sensor can be used to measure different physical parameters including strain (Tan et al., 2008)/stress (Pereles et al., 2014), liquid density and viscosity (Jain et al., 2001), fluid flow velocity (Pereles et al., 2009) and the elastic modulus of thin films (Liang et al., 2007). Additionally, the resonant frequency of the ME sensor is sensitive to the mass change. The ME sensors modified with a chemical or biological sensing membrane can be used to detect a variety of bacteria, such as *Escherichia coli* (Ruan et al., 2003), *Bacillus anthracis* spores (Xie et al., 2014), *Salmonella typhimurium* (Park et al., 2013) and *Staphylococcal enterotoxin* B (Ruan et al., 2004).

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In this study, we have developed a detection system consisting of a test paper and a measurement circuit for the detection of CSFV. The measurement circuit is designed based on the impedance analysis technique. The test paper is fabricated by immobilizing an ME biosensor in a rectangular insulation supporting frame. The antibody against the CSFV is coated onto the ME biosensor surface through physical absorption. To evaluate the performance, test papers were immersed in the CSFV suspensions at different concentrations. The scanning electron microscopy (SEM), sandwich-based ELISA and the reference biosensor approach were separately used to confirm that the response signal is from the antibody-antigen binding of CSFV on the biosensor surface.

2. Theory and principle

Under the superimposition of both alternating and static magnetic fields, the ME sensor vibrates longitudinally. When the sensor vibrates at its resonance frequency, the conversion of the magnetic energy into elastic energy reaches a maximum. The resonance frequency (f_0) of the sensor with length L, density ρ, elastic module E, and Poisson's ratio v is given by Eq. (1) (Baimpos et al., 2012).

$$f_0=\frac{1}{2L}\sqrt{\frac{E}{\rho\left(1-v^2\right)}} \tag{1}$$

The resonance frequency of the ME sensor is sensitive to the mass change. For a small extra mass load of Δm on the sensor of mass M ($\Delta m \ll M$), the change in the resonance frequency (Δf) is given by Eq. (2) (Hiremath et al., 2015; Schmidt and Grimes, 2001).

$$\frac{\Delta f}{\Delta m}=-\frac{f_0}{2M} \tag{2}$$

From Eq. (2), it is clear that an extra mass load on the sensor surface results in a decrease in f_0. The anti-CSFV IgG is immobilized on the surface of the ME sensor to selectively adsorb the CSFV. Due to the combination of the antibody and antigen, the extra mass load on the biosensor increases, which in turn induces a decrease in the resonance frequency. In this way, the CSFV can be detected by monitoring the resonance frequency of the biosensor.

3. Detection system design

3.1. Measurement circuit design

Over the past years, three detecting methods have been developed to monitor the resonance frequency of the ME sensor, namely the time-domain measurement technique (Zeng et al., 2002), the frequency-domain measurement technique (Zeng et al., 2005) and the impedance de-tuning technique (Grimes et al., 1999). In the time-domain measurement technique, the ME sensor is excited by a sinusoidal magnetic field generated by a passing current through a coil. The sensor is interrogated by a pulse-like excitation signal, and the resonance frequency of the sensor is subsequently calculated by counting the number of oscillations per sensor ring-down time or using a Fast Fourier transform (FFT). The mechanism of the frequency-domain measurement technique consists in generating a sweep steady-state signal, and the response of the ME sensor is measured at every frequency point. The sensor resonance frequency is determined as the frequency at which the maximum response, such as the greatest output amplitude, is obtained. Due to its attributes of easy implementation and simple operation process, the impedance de-tuning technique is adopted in our design.

3.1.1. Impedance de-tuning technique principle

An ME sensor is inserted inside a solenoid coil, and an AC excitation signal is applied to the coil. A time varying magnetic field is generated inside the coil which drives the sensor to oscillate, with the vibration amplitude and phase being functions of the excitation frequency and impedance. Since the permeability of the sensor increases significantly at resonance, a sharp peak occurs at the resonance frequency of the solenoid's impedance spectrum. Thus, the resonance frequency of the sensor can be obtained by measuring the frequency at the sharp peak, as shown in Fig. S1.

3.1.2. Measurement circuit

The circuit based on the impedance analysis technique to characterize ME sensors was developed in a previous study (Zeng et al., 2006). For such a circuit, we made some improvements to certain elements, such as the new microcontroller that has a 12-bit multi-channel analog to digital converter on the chip, LCD display and USB communication interface. The block diagram of the circuit design in this study is shown in Fig. S1. The key components of the detector include the following:

Microcontroller: A STM32F103VET6 microcontroller (the ARM microcontroller family from STMicroelectronics Corporation) is chosen for its efficient bit manipulation and large embedded memory (512 KB of flash memory, 64 KB of SRAM). It has three 12-bit analog to digital converters on the chip and can work at frequencies up to 72 MHz. In this circuit, an 8 MHz crystal clock is used.

Direct digital synthesis (DDS): The DDS chip, AD9851, is used to digitally synthesize a sine wave of numerically controlled frequency from a reference clock. It is serially interfaced with the microcontroller. The crystal clock used in the DDS circuit is 30 MHz and provides a synthesized wave with a frequency resolution of 2 Hz.

RMS-to-DC converter: An RMS-to-DC converter, AD536A, is used to compute the true root-mean-square (RMS) value of the AC signal. It is a complete monolithic integrated circuit which performs a true RMS-to-DC conversion. The AD536A has a 450 kHz bandwidth and performs the RMS-to-DC conversion with the aid of an external capacitor.

DC biasing: A magnet is used to provide a DC biasing magnetic field that turns the sensor to an optimal operational point, facilitating sensor excitation and detection.

USB communication interface: The USB interface is adopted to communicate with the computer. The test data stored in the detector can be copied to the computer through such interface.

LCD display: An LCD is chosen to immediately display the impedance spectrum curve and the test resonance frequency value.

3.2. Test paper design

3.2.1. Preparation of the ME sensor platform

The ME strip-shaped sensor platform composed of the Metglas alloy 2826 ($Fe_{40}Ni_{40}P_{14}B_6$) was purchased from Honeywell Corporation (Morristown, NJ, USA), and cut into 5 mm × 1 mm × 0.028 mm using a computer-controlled laser cutting machine. The sensor platform was ultrasonically cleaned in ethanol for 15 min and rinsed with deionized water, to remove any debris and organic film on the sensor surface, and then dried in a stream of nitrogen. To protect the sensor platform from corrosion and to promote the immobilization of the antibody, both sides of the cleaned sensor platform were sputtered with chromium (100 nm thick), followed by gold (100 nm thick). The middle chromium layer enhances the adhesion between the gold and the sensor platform. The gold-coated sensor platform were annealed in a vacuum oven at 220 °C for 2 h to eliminate any residual stress in the sensor and improve adhesion of the gold layer to the ME