LabVIEW-based impedance biosensing system for detection of avian influenza virus

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Abstract: In order to detect the multiple avian influenza viruses (AIVs) rapidly, specifically and sensitively, a LabVIEW and microelectrode array-based impedance biosensor was developed and demonstrated. A laptop with LabVIEW software was used to generate excitation signals at different frequencies with an audio card and measure the impedance of target viruses through a data acquisition card. The audio card of the laptop was used as a function generator, while a data acquisition card was used for data communication. A virtual instrument was programmed with LabVIEW to provide a platform for impedance measurement, data processing, and control. Six interdigitated microelectrodes were placed at the bottom of six wells on a microplate to form six sensors for different AIVs and controls. Then, AIV specific ligands were immobilized on the microelectrode surface to capture target viruses. To enhance the sensitivity, AIV specific aptamers conjugated gold nanoparticles and thiocyanuric acid were employed to form a network structure and used as an amplifier. Results of the measured impedance were compared with a commercial IM6 impedance analyzer, and the error was less than 5%. The developed biosensor was portable with the sensitivity and specificity for applications to on-site or in-field rapid screening of avian influenza viruses.

Keywords: biosensor, impedance detection, avian influenza virus, LabVIEW

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1 Introduction

Potential pandemic outbreak of human infection with AIV H5N1 or H7N9 can cause a serious health threat worldwide. The Highly Pathogenic Avian Influenza (HPAI) H5N1 virus, which originally emerged in Southeast Asia at late 1990’s, cost the poultry industry an estimated $10 billion between 1997 and 2008. Since 2003, there have been 398 deaths among 676 cases of human infection caused by H5N1, for a mortality rate of 59%[1]. In late February and early March of 2013, the AIV H7N9 emerged in humans in Shanghai and surrounding provinces of China. As of May 29, 2013 there were reported 132 confirmed H7N9 infections with 37 fatalities[2]. Rapid, sensitive and specific detection of AIVs is becoming increasingly important and urgent. The technology for diagnosing AI infections is available, such as viral culture, diagnostic test kits, RT-PCR and ELISA methods, but these tests are of some shortcomings, such as poor specificity, low sensitivity, time consuming and high costs, and require a laboratory and a highly trained technician. In a report of WHO (2006)[3], it was pointed out that “A simple, rapid, robust and reliable test, suitable for use in the field or at the patient’s bedside, is urgently needed”. Considerable effort has been directed towards the development of simple biosensors for the
detection of AIV, such as surface plasmon resonance, quartz crystal microbalance, fluorescence, optical interferometry, imaging ellipsometry and electrochemistry [4-9]. These developed biosensors have shown potential but are not suitable for rapid, in-field testing. Therefore, the goal of this study was to develop a portable impedance biosensing system with the sensitivity and specificity for applications to on-site or in-field rapid screening of multiple AIVs.

Impedance measurement/detection is a powerful detection method, ideal for easy integration into multi-array or microprocessor-controlled diagnostic tools and incorporation into miniaturized analytical systems. Impedance measurement/detection can be used for “in field” measurements in combination with a portable measuring unit, and can also provide real-time in situ monitoring[10]. In recent years, with the rapid development of computer technology, the virtual instrument technology has been widely used. In this study, virtual instrumentation using LabVIEW with a laptop was employed to generate excitation signals at different frequencies with an audio card and measure the impedance of target viruses through a data acquisition card. The developed portable biosensing system with interdigitated microelectrode based microplate array and virtual instrumentation based impedance measurement platform, is suitable for real time detection of AIVs in the field.

2 Materials and methods

2.1 Materials

Inactivated AIV H5N1 was supplied by USDA APHIS Research Laboratory (Ames, IA). The selected aptamer with high affinity and specificity against AIV H5N1 surface protein was developed in our group with detailed information described in our previous study[11]. Biotin labeled aptamer was obtained from Integrated DNA Technologies, Inc. (Coralville, IA). The sequence is 5'-Biotin-GTG TGC ATG GAT AGC ACG TAA CGG TGT AGT AGT AAC GTG CGG GTA GGA AGA AAG GGA AAT AGT TGT CGT GTT G-3'. All chemicals used in this study unless specified were purchased from Sigma-Aldrich (St. Louis, MI).

2.2 Interdigitated microelectrode and microplate

Interdigitated microelectrodes were fabricated by Institute of Semiconductor of Chinese Academy of Sciences (Beijing, China). A 100 nm thick gold layer was sputtered on a cleaned glass substrate. In order to improve the adhesion of the gold to substrate, a 10 nm thick Cr layer was deposited prior to the gold layer. The microelectrodes were then patterned by mask photolithography and metal etching processes. Figure 1 shows the interdigitated microelectrode and the array in a microplate used in the test. Six interdigitated microelectrodes were placed at the bottom of six wells on a microplate to form six sensors for different AIVs and controls.

![Figure 1](image_url)

Figure 1  A: Interdigitated microelectrode; B: Microelectrode array on a microplate: (a) top view and (b) bottom view

2.2.1 Procedure

The whole experimental procedure contains four steps: electrode cleaning, surface immobilization, AIV virus detection, and signal amplification. Step 1: The microelectrode was cleaned by immersing in 1 mol/L NaOH for 5 min, rinsed with deionized water, immersed in 1 mol/L HCl for 2 min, rinsed with deionized water, and then wiped gently with alcohol wetted lens paper. After final rinsing with deionized water, the microelectrode was dried with a stream of nitrogen. Step 2: The clean microelectrode was incubated with 50 µL of streptavidin (0.5 mg/mL) at room temperature for 45 min. Following the incubation, the microelectrode was rinsed with deionized water and dried with nitrogen stream, and then incubated with 2 µg/mL biotin-aptamer (50 µL) for 30 min at room temperature.
After rinsing with deionized water and drying with nitrogen, the microelectrode was incubated with 0.1 mg/mL PEG (poly (ethylene glycol) methyl ether thiol) (50 µL) as a blocking solution at room temperature for 30 min. After another rinsing with deionized water and drying with nitrogen stream, the microelectrode was ready for use in detection tests. Step 3: AI detection was performed by dropping 50 µL of inactivated AI H5N1 virus onto the microelectrode surface coated with the aptamer, and incubating it at room temperature for 45 min. The impedance change caused by the target AI virus was calculated by subtracting the impedance measured at the end of step 3 from the impedance measured at the end of step 2. Step 4: 50 µL of TCA+NPApt (a conjugation of aptamer, gold nanoparticles and thiocyanuric acid) was added and incubated for 40 min at room temperature for further amplification of the signal.

2.2.2 Structure of biosensor and impedance measurement

The structure and main components of the biosensor are shown in Figure 2. Two channels sine signal with different frequencies were generated by LabVIEW through the sound card of laptop. As the signal source power, they were added to the circuit series connection of a changeless resistor $R$ and an unknown component $U$ (Figure 3). When the values of voltages $V_1$ and $V_2$ were tested by LabVIEW through the DAQ card, we can calculate the impedance $Z$ of the unknown component with Equations (1) and (2).

$$Z = \frac{V_2}{I} = \frac{V_2}{V_1/R}$$

The diagram of the program based on LabVIEW is shown in Figure 4. Signal generator program is indicated in Figure 4a. In order to ensure the output signal’s amplitude, a while loop of auto adjustment was designed (Figure 4b). Data acquisition and display were constructed using comprehensive library of VIs and example programs for NI LabVIEW (Figure 4c). Polymorphism Library Functions were used in this program. The front panel of the virtual instrument indicates the excitation signal, impedance recording and the final report of the detected AVIs (Figure 5).

3 Results and discussion

3.1 Principle of the biosensing of AIV

The principle of the developed biosensor is illustrated in Figure 6. Briefly, AIV specific ligands were pre-coated on the microelectrodes. The target AIVs were then captured by the immobilized ligands. To enhance the sensitivity, AIV specific aptamer was conjugated with gold nanoparticles and thiocyanuric acid in a sandwich format and then used as an amplifier (TCA+NPApt). The gold nanoparticle offered excellent electrochemical signal transduction, and thiocyanuric acid (a star-shaped trithiol molecule) was an effective capping agent for the formation of the network structure. The network-like thiocyanuric acid/gold nanoparticles can be used for signal amplification. It is due to the electrochemical oxidation/reduction of gold nanoparticles of AuCl$_4^-$.

3.2 Proof of the concept for AIV detection

Inactivated AI H5N1 virus with titers in the range from $2^0$ to $2^5$ HAU in buffer solution was tested using the developed biosensor system. Figure 7 shows the typical Nyquist diagram of impedance spectra on the bare microelectrode, streptavidin modification, aptamer immobilization, PEG blocking, H5N1 binding ($2^3$ HAU) and signal amplification with [Fe(CN)$_6$]$_3^{-}/4^{-}$ as a redox probe in the frequency range from 1 Hz to 1 MHz.

The result indicated that the developed impedance biosensor with specific aptamer was feasible for detection of AIVs. The result also demonstrated that TCA+NPApt
(a conjugation of aptamer, gold nanoparticles and thiocyanuric acid) could be used as an amplifier for further amplification of the binding reaction between the aptamer and target AIV.

Figure 4  LabVIEW Program for the impedance biosensing system

Figure 5  VI front panel for impedance measurement using LabVIEW
3.3 Calibration of impedance measurement

In order to check the detection accuracy of the developed system, the performance of solid circuit was tested by detection of solid capacity under different frequency and then in comparison with calculation. The result is listed in Table 1. After measuring the impedance of constant capacitor and its capacitance, it demonstrated that the difference of capacitance calculated under different frequencies was not significant, with an error less than 5%. The precision of the difference value was tested. The result was very close to the true value, when the sample period was setup to 1 min. As shown in Figure 5, the maximum error in the whole range configured was less than 50 Ω.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>Voltage 1 (V)</th>
<th>Voltage 2 (V)</th>
<th>Capacity (F)</th>
<th>Error (%)</th>
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<tr>
<td>10</td>
<td>0.169</td>
<td>0.961</td>
<td>10 713 159.76</td>
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</tr>
<tr>
<td>20</td>
<td>0.333</td>
<td>0.914</td>
<td>10 342 198.2</td>
<td>9.66912E-08</td>
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<tr>
<td>30</td>
<td>0.455</td>
<td>0.862</td>
<td>10 707 745.05</td>
<td>9.35903E-08</td>
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<tr>
<td>40</td>
<td>0.566</td>
<td>0.788</td>
<td>10 491 816.25</td>
<td>9.53124E-08</td>
</tr>
<tr>
<td>50</td>
<td>0.663</td>
<td>0.71</td>
<td>10 087 782.81</td>
<td>9.91298E-08</td>
</tr>
<tr>
<td>60</td>
<td>0.72</td>
<td>0.671</td>
<td>10 534 700.00</td>
<td>9.49244E-08</td>
</tr>
<tr>
<td>70</td>
<td>0.776</td>
<td>0.611</td>
<td>10 383 850.52</td>
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<tr>
<td>80</td>
<td>0.811</td>
<td>0.564</td>
<td>10 481 637.48</td>
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</tr>
<tr>
<td>90</td>
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<td>0.53</td>
<td>10 775 395.68</td>
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<tr>
<td>100</td>
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<td>0.482</td>
<td>10 486 004.62</td>
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<td>0.186</td>
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<tr>
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<tr>
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<td>0.097</td>
<td>11 008 915.66</td>
<td>9.08355E-08</td>
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</table>

In addition, the measured impedance by the developed biosensor was compared with that by an impedance analyzer (BAS, IM6, West Lafayette, IN) and the result (Figure 8) indicated that both curves matched well in the range of frequencies from 50 Hz to 5000 Hz with a correlation coefficient of 0.93. This system could accurately control the frequency and amplitude of the excitation signal. The accuracy of the system could be further enhanced if a DAQ with more bits is selected, the interference is better shielded, and/or the algorithm is optimized. Figure 8 shows that the system has a large error at low frequency, but the fitting effect is particularly good when the frequency exceeds 50 Hz. In practical applications, there are very few cases of lower than 50 Hz, such as the range of the frequency bands used in this study is 60-200 Hz.

3.4 Repeatability of impedance measurement

Two groups of impedance measurement tests were conducted using the same microelectrode with two methods and their results were compared. The impedance was measured in the presence of 10 mM \( \text{[Fe(CN)₆]}^{3-/4-} \) (1:1) mixture in PBS (pH7.4) as a redox probe in the frequency range from 50 Hz to 225 Hz with an amplitude of 5 mV. Bode (impedance and phase vs...
An impedance biosensing system was successfully designed in this research as a compact, portable instrument with the sensitivity and specificity for on-site or in-field rapid screening of multiple AIVs in poultry samples. The results showed that this portable virtual instrument based impedance analyzer has a high accuracy and an acceptable repeatability. Calibration algorithm was optimized for this virtual instrument based on the comparison with the results of IM6. The measurement error was limited less than 5%.

The developed impedance biosensing system provided a novel platform for development of biosensing instruments for in-field rapid detection of pathogenic viruses and bacteria in agriculture and food.

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**References**


