Biosensors and Bioelectronics 38 (2012) 67–73

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Rapid detection of avian influenza H5N1 virus using impedance measurement of immuno-reaction coupled with RBC amplification

Jacob Lum [a,b], Ronghui Wang [c], Kentu Lassiter [b], Balaji Srinivasan [d], Daad Abi-Ghanem [e], Luc Berghman [e], Billy Hargis [a,b], Steve Tung [d], Huaguang Lu [f], Yanbin Li [a,b,c,*]

[a] Cell and Molecular Biology Program, University of Arkansas, Fayetteville, AR 72701, USA
[b] Department of Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA
[c] Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, AR 72701, USA
[d] Department of Mechanical Engineering, University of Arkansas, Fayetteville, AR 72701, USA
[e] Departments of Poultry Science and Veterinary Pathobiology, Texas A&M University, College Station, TX 77843, USA
[f] Animal Diagnostics Laboratory, Pennsylvania State University, University Park, PA 16801, USA

ARTICLE INFO

Article history:
Received 28 March 2012
Accepted 30 April 2012
Available online 8 May 2012

Keywords:
Avian influenza
AI H5N1
Impedance measurement
Interdigitated microelectrode
Immunosensor
Virus detection

ABSTRACT

Avian influenza virus (AIV) subtype H5N1 was first discovered in the 1990s and since then its emergence has become a likely source of a global pandemic and economic loss. Currently accepted gold standard methods of influenza detection, viral culture and rRT-PCR, are time consuming, expensive and require special training and laboratory facilities. A rapid, sensitive, and specific screening method is needed for in-field or bedside testing of AI virus to effectively implement quarantines and medications. Therefore, the objective of this study was to improve the specificity and sensitivity of an impedance biosensor that has been developed for the screening of AIV H5. Three major components of the developed biosensor are immunomagnetic nanoparticles for the separation of AI virus, a microfluidic chip for sample control and an interdigitated microelectrode for impedance measurement. In this study polyclonal antibody against N1 subtype was immobilized on the surface of the microelectrode to specifically bind AIV H5N1 to generate more specific impedance signal and chicken red blood cells (RBC) were used as biolabels to attach to AIV H5N1 captured on the microelectrode to amplify impedance signal. RBC amplification was shown to increase the impedance signal change by more than 100% compared to the protocol without RBC biolabels, and was necessary for forming a linear calibration curve for the biosensor. The use of a second antibody against N1 offered much greater specificity and reliability than the previous biosensor protocol. The biosensor was able to detect AIV H5N1 at concentrations down to 10^3 EID_{50} ml^{-1} in less than 2 h.

1. Introduction

Avian influenza viruses (AIV) are a considerable health problem for both humans and animals. One AIV subtype, H5N1, has been shown to infect wild birds, domestic poultry and mammals, including humans, with deadly effects. First discovered in 1997 in Hong Kong, H5N1 has spread throughout much of south Asia and parts of Europe and Africa (Peiris et al., 2007). The virus has a high mortality rate in both poultry and humans, often having a 100% mortality rate in poultry flocks and a 60% mortality rate in humans, with 587 reported human cases and 346 deaths since 2003 (WHO, 2011). In addition to the health impact of AIV H5N1, the possible economic impact stands to be massive. Already AIV H5N1 is estimated to have cost the poultry industry over $10 billion dollars and the World Bank has estimated that a severe outbreak in humans would cost upwards of $3.13 trillion to the global economy (Burns et al., 2008). Rapid detection and identification of H5N1 is crucial to controlling outbreaks (MacKay et al., 2008). Definitive answers from a laboratory may take 2–3 days, during which time the virus may be allowed to spread. For this reason, a rapid, specific and in-field method of detection for AIV H5N1 is needed.

Current gold standard methods of AIV detection, viral isolation culture and rRT-PCR, are time-consuming, expensive and require special training and facilities (Charlton et al., 2009; Ellis and Zambon, 2002). Other techniques of AIV detection, such as ELISA and immunochromatographic strips, lack the specificity and sensitivity required. Biosensors, which combine a biological sensing element, a transducer, and a signal processing unit, have shown promise in the fields of food safety, drug development, environmental monitoring, healthcare, and pathogen detection

* Correspondence to: Department of Biological and Agricultural Engineering, University of Arkansas, 203 Engineering Hall, Fayetteville, AR 72701, USA. Tel.: +1 4795752881; fax: +1 4795752846.
E-mail address: yanbinli@uark.edu (Y. Li).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.04.047

(Amano and Cheng, 2005). Several types of biosensors, such as surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and optical interferometric, have been researched as alternatives to conventional detection methods for avian influenza virus, but while the developed biosensors have shown potential, they lack subtype specificity and many are not practical for use in the field (Estmer-Nilsson et al., 2010; Sato et al., 1996; Peduru Hewa et al., 2009; Xu et al., 2007).

Impedance biosensors, which rely on electrochemical changes in the electrode environment to detect an analyte, have advantages over traditional detection methods of AIV due to their simple design, relatively low cost and ease of miniaturization (Pejcic et al., 2006). Impedance biosensors can be combined with interdigitated microelectrode arrays (IDAMs) to overcome many of the problems that occur with macroelectrodes. IDAMs offer the desired features of low ohmic drop, fast establishment of steady-state, rapid reaction kinetics, increased signal to noise ratio, small sample sizes and reduced detection time due to their low response time (Varshney and Li, 2009). The use of microfluidic devices in biosensors has also shown promise in the field of pathogen detection, offering a high surface-to-volume ratio, the ability to precisely control small volumes of sample and lower detection time (Yager et al., 2006). When coupled with microbial detection methods, immunomagnetic separation can provide several advantages over traditional isolation techniques. Immunomagnetic separation is simple, provides high capture efficiency and specificity, can be used to concentrate a sample for more sensitive detection and requires no expensive equipment or special training (Horak et al., 2007).

A Faradic impedance biosensor for the detection of AIV was studied using an open interdigitated array microelectrode with immobilized polyclonal antibody against H5 and RBC amplification (Wang et al., 2009). The developed biosensor had a lower detection limit of 10^3 EID_{50}/ml and was only specific to the H5 antigen. Another biosensor system was developed for AIV detection using a microfluidic biochip with an embedded microelectrode array combined with immunomagnetic separation (Li et al., 2006; Wang et al., 2007). In their research anti-H5 antibody-coated nanobeads were used to isolate the virus and the impedance of the sample was measured using the microfluidic biochip with no antibody immobilized to the microelectrode. Their result showed that the lower detection limit was 10^3 EID_{50}/ml for detection of AIV H5.

In this study, we describe an improved non-Faradic impedance biosensor using a second antibody against N1 subtype, RBC biolabels and an interdigitated microelectrode array embedded in a microfluidic biochip for the specific detection of AIV H5N1. A sample of AIV was isolated using immunomagnetic nanobeads coated in monoclonal antibody against H5. The microelectrode surface was modified using Protein A (*Staphylococcus aureus*) and then polyclonal antibody against N1 was immobilized. Target AIV H5N1 was bound to the antibody on the microelectrode surface, causing a change in impedance compared to a control sample. RBCs were used as biolabels to amplify the impedance change through their binding to the AIV on the microelectrode. RBCs were used as biolabels to amplify the antibody-virus binding due to their larger diameter (7–12 μm) compared to the virus (80–120 nm), and strong and specific binding by virus hemagglutinin to sialic acid linkages found on the cell surface (Cell Size Database, 2012; Suarez and Schultz-Cherry, 2000; Lamb and Krug, 2001; Murphy and Webster, 1996). Both the virus and the RBC act as resistors in the system, and the RBC has a larger resistive value due to its larger size compared with the virus. Non-target influenza subtypes were used to test the specificity of the biosensor. Transmission electron microscopy and atomic force microscopy were used to confirm the binding of AIV H5N1 and RBCs.

2. Materials and methods

2.1. Materials

Phosphate buffered saline (PBS, 10 mM, pH 7.4), bovine serum albumin (BSA) and Protein A (*S. aureus*) were purchased from Sigma-Aldrich (St. Louis, MI). Protein A and BSA were both reconstituted in PBS. Washing solution (20 ×; 0.04M imidazole buffered saline with 0.4% Tween 20) was purchased from KPL Inc. (Gaithersburg, MD) and diluted with Milli-Q water (Milli-Q, 18.2 MΩ cm, Bedford, MA) to 1:200,000 dilution for use as a measuring buffer. Monoclonal mouse antibody against H5 and polyclonal rabbit antibody against N1 were provided by Dr. Luc Berghman's research laboratory (Department of Poultry Science, Texas A&M University). Chicken red blood cells suspended in isotonic dextrose at a concentration of 0.5% (w/v) were obtained from the Poultry Health Lab (University of Arkansas, Fayetteville, AR). Magnetic streptavidin-coated 30 nm nanobeads were acquired from Ocean NanoTech (Springdale, AR). The nanobeads were used at a concentration of 0.5 mg ml^{-1}.

Avian influenza A/H5N1 (Scotland 59) was provided by the USDA/APHIS National Veterinary Services Lab (Ames, IA). The virus was inactivated by the USDA with β-propiolactone, eliminating viral infectivity while preserving hemagglutination activity (Goldstein and Tauraso, 1970). The stock concentration of the virus was 10^7 EID_{50} ml^{-1} or the equivalent HA titer of 128. Non-target influenza virus subtypes were provided by Dr. Huaguang Lu (Animal Diagnostics Laboratory, Pennsylvania State University, University Park, PA). All non-target virus subtypes were used at an HA titer of 128.

2.2. Microfluidics biochips with embedded interdigitated microelectrodes

A microfluidics biochip with an embedded gold interdigitated array microelectrode was fabricated by Dr. Steve Tung's laboratory (University of Arkansas, Fayetteville, AR). A microfluidic channel (40 μm deep and 100 μm wide) with an oval-shaped microfluidics chamber (40 μm deep, 500 μm wide and 1723 μm long; 34.5 nl volume) was molded from PDMS and fixed to an interdigitated microelectrode chip. Each electrode consisted of 25 pairs of 10 μm wide electrode fingers spaced 10 μm apart.

2.3. AIV H5 separation with antibody-coated 30 nm magnetic nanobeads

Monoclonal mouse antibody to H5 was biotinylated using sulfo-NHS-biotin and purified using a Slide-A-Lyzer dialysis kit from Pierce Protein Research Product (10K MWCO, Rockford, IL). The antibody was diluted to a concentration of 260 μg ml^{-1}. Thirty-three microliters of 30 nm magnetic nanobeads (0.5 mg ml^{-1}) was washed with 250 μl of PBS and resuspended in 67 μl of PBS. The nanobeads were mixed with 67 μl of the antibody for 30 min in a rotating mixer. The nanobead-antibody conjugates were separated using a magnet for 30 min, washed with 150 μl of PBS and resuspended in 67 μl of PBS. The conjugates were added to 67 μl of virus sample and incubated for 45 min at 37 °C, forming nanobead–antibody–AIV complexes. The complexes were separated with a magnet for 1 h and the rest was removed. The complexes were washed with 150 μl of measuring buffer and separated with a magnet for 1 h and the waste was removed. The complexes were resuspended in 150 μl of measuring buffer and stored at 4 °C for further impedance measurement.