Biosensors and Bioelectronics 100 (2018) 348–354

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Rapid detection and subtyping of multiple influenza viruses on a microfluidic chip integrated with controllable micro-magnetic field

Rui-Qiao Zhang[1], Shao-Li Hong[1], Cong-Ying Wen, Dai-Wen Pang, Zhi-Ling Zhang*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, State Key Laboratory of Virology, Wuhan University, Wuhan 430072, PR China

ARTICLE INFO

Keywords:
Microfluidic chip
Magnetic bead
Influenza virus
SA-QDs
DNA

ABSTRACT

Influenza viruses have threatened animals and public health systems continuously. Moreover, there are many subtypes of influenza viruses, which have brought great difficulties to the classification of influenza viruses during any influenza outbreak. So it is crucial to develop a rapid and accurate method for detecting and subtyping influenza viruses. In this work, we reported a rapid method for simultaneously detecting and subtyping multiple influenza viruses (H1N1, H3N2 and H9N2) based on nucleic acid hybridization on a microfluidic chip integrated with controllable micro-magnetic field. H1N1, H3N2 and H9N2 could be simultaneously detected in 80 min with detection limits about 0.21 nM, 0.16 nM, 0.12 nM in order. Moreover, the sample and reagent consumption was as low as only 3 μL. The results indicated that this approach possessed fast analysis and high specificity. Therefore, it is expected to be used to simultaneously subtype and detect multiple targets, and may provide a powerful technique platform for the rapid detection and subtyping analysis of influenza viruses.

1. Introduction

In recent years, influenza A infections have become one of the most prevalent diseases, and caused highly infectious respiratory illness in humans, poultries, birds, and other mammals, which have brought devastating effects on social and economic development, and threatened human health and life continuously (Tharakaraman and Sasisekharan, 2015). For instance, the Spanish flu pandemic in 1918 (influenza A/H1N1) caused 50–100 million people deaths worldwide (Johnson and Mueller, 2002). The Asian flu pandemic of 1957 (influenza A/H2N2) caused estimated 2 million fatalities (Joseph et al., 2015). In 1968, the Hong Kong flu pandemic (influenza A/H3N2) killed one million people worldwide (Cox and Subbarao, 2000). Most recently, the Swine Flu outbreak (a novel H1N1 virus) in 2009 has infected people and quickly spread worldwide causing serious health concerns (Smith et al., 2009). In the spring of 2013, a novel reassortant avian-origin influenza A (H7N9) virus caused human infections (Gao et al., 2013). Since then, H7N9 has caused many people infections with a death rate over 30% (Yan et al., 2016). As known, influenza A (H9N2) viruses in poultry have occasionally been passed on to humans and other mammals (Liu et al., 2016). What's more, different types of influenza viruses have been observed to spread simultaneously or individually in specific time periods and particular regions, and clinical laboratory test to determine the type of influenza virus has become an important part of the epidemiological investigation and epidemic prevention. Therefore, rapid and precise detection and subtyping of the influenza virus during the early stage of infection are needed not only to provide an immediate and accurate diagnosis, but also to prevent its spread timely.

Traditionally, various types of diagnostic methods have been used for surveillance of influenza infection, such as viral culture (Chan et al., 2013; Swayne et al., 2007), fluorescence immunoassay (FIA) (Shibata et al., 2009), enzyme-linked immunosorbent assay (ELISA) (Laurie et al., 2012), and real-time fluorescence quantitative reverse transcription- polymerase chain reaction (RFQ-RT-PCR) (Hindiyeh et al., 2005). Viral culture followed by physiological observations of the cultured cells, eggs, or laboratory animals is the acknowledged standard detection method (Chan et al., 2013). However, this method requires a relatively long-term culturing time, labor-intensive operations and well-trained technicians. Serological assays such as FIA and ELISA are based on the detection of color change or fluorescence labeled on antibody involving a series of tedious processes such as incubation and washing steps, which also are time-consuming (over 4 h), labor-intensive and require well-trained technicians (Laurie et al.,

* Corresponding author.
E-mail address: zlzhang@whu.edu.cn (Z.-L. Zhang).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2017.08.048
Received 21 June 2017; Received in revised form 10 August 2017; Accepted 21 August 2017
Available online 18 September 2017
0956-5663/

2012). Alternatively, as the well-recognized molecular diagnostic assays, traditional polymerase chain reaction (PCR) and real-time fluorescence quantitative reverse transcription PCR (RFQ-RT-PCR) provide highly sensitive and selective protocols for diagnosis of influenza infection. However, these molecular diagnostic assays require relatively expensive instruments and reagents, and also are labor-intensive due to the complicated and multistep nucleic acids extraction, which prohibit the on-site and real-time practical applications.

Combining microfluidic device with nanomaterials may overcome these difficulties mentioned above (Zhang et al., 2013). The integrated microfluidic device permits low sample and reagent consumption, flexible liquid manipulation in very low volumes, and process integration (i.e., sample separation, enrichment, reaction, and detection) (Tseng et al., 2016; Xia et al., 2016; Yu et al., 2013), which greatly saves the detection time and makes point-of-care test possible. Nanomaterials provide controllable reaction carrier and/or excellent fluorescence labeling for pathogen diagnosis, which greatly improves the sensitivity, and manipulability of diagnosis. Among of them, magnetic nanomaterials with high magnetic susceptibility and low loss rate during the treatment process have been extensively used for the separation and enrichment of bacteria, viruses, and cells (Lee et al., 2014; Song et al., 2011; Wen et al., 2014; Wu et al., 2015; Yen et al., 2013). Quantum dots (QDs), as fluorescent semiconductor nanocrystals, owning to their unique photochemical stability and high photoluminescence quantum yields, have also been widely applied in fluorescence labeling (Medintz et al., 2005; Reschgenger et al., 2008), in-vivo bioimaging (Chen et al., 2015; Gu et al., 2012) and pathogen detection (Wu et al., 2015; Zhang et al., 2013; Zhang and Wang, 2012; Zhao et al., 2012).

In this study, we combined integrated microfluidic chip with magnetic nanoparticles and QDs to accomplish detection and subtyping of multiple influenza viruses (H1N1, H3N2, H9N2) simultaneously. This microfluidic chip possessed three branch channels for multiple detections, and integrated with a controllable micro-magnetic field and a heating region. The integrated micro-magnetic field on chip was used to capture magnetic nanoparticles modified with capture probe DNAs (CP-DNAs) in each channel to form a magnetic reaction area for recognizing the corresponding target cDNA sequences (T-DNAs) of influenza A viruses and the following detection. The capture probe DNAs (CP-DNAs) and the report probe DNAs (RP-DNAs) were complementary to the corresponding T-DNAs and formed a sandwich structure, and high-luminance quantum dots (QDs) conjugated with streptavidin (SA-QDs) were bonded to the sandwich structure by the biotin modified on the report probe DNAs. The sandwich-structured complexes were real-time measured by an Olympus IX70 inverted fluorescence microscope equipped with EM-CCD DU885K-C00-500. This design realized the rapid detection and subtyping of multiple influenza viruses with high sensitivity. So this approach may provide a powerful technique platform for the rapid multiple synchronous detection and subtyping analysis of pathogens.

2. Experimental

2.1. Reagents and materials

AZ50XT photoresist (PR) and AZ4620 were obtained from AZ Electronic Materials (AZ Electronic Materials Corp., USA). The silicon wafers were purchased from the Institute of Microelectronics of Chinese Academy of Sciences. Indium tin oxide (ITO) glasses were purchased from Lai Bao Hi-Tech Co., Ltd. Polydimethylsiloxane (PDMS) and curing agent were purchased from GE (GE Toshiba Silicones Co., Ltd., Japan). High purity silver paint was purchased from SPi Supplies. N-hydroxysuccinimide (NHS) and N-Ethyl-N′-(3-dimethyl-aminopropyl)- carbodiimide hydrochloride (EDC) were purchased from Sigma. Superparamagnetic beads (SMB) (MasterBeads Carboxylic Acid 0215, 500 nm diameter) were obtained from AdemTech (Pessac, France). QDs 605 streptavidin conjugate (SA-QDs) were purchased from Wuhan Jiayuan Quantum Dots Co., Ltd. Ultrapure water (18.2 MΩ·cm) supplied by a Millipore water-purification system (Synergy UV, Millipore, USA) was used to prepare buffer solutions. Target cDNA of H1N1, H3N2, H9N2, capture probe DNA, report probe DNA, and Random DNA were obtained from Invitrogen Corp. And the sequences have showed following:

Target cDNA sequences of H1N1, H3N2, H9N2:

T-H1: 5′-ATT CAA TCC AGA GGT CTA TTT GGA GCC ATT GCC GGT TTT ATT GAA-3′

T-H3: 5′-TAT GCC ACC CTT AGG TCA CTA GTT GCC TCA TCT GGC AAC CTG GAA-3′

T-H9: 5′-TAG AAG GGG TCA AGC TGG AAT CTG AAG GAA CTT ACA AAA TCC TCA-3′

Amine-modified capture probe DNA sequence:

CP-H1: 5′-NH_2-$(CH_2)_{12}$-TTC AAT AAA ACC GGC AAT GG-3′

CP-H3: 5′-NH_2-$(CH_2)_{12}$-TTC CAG GTT GCC AGA TGA GGC A-3′

CP-H9: 5′-NH_2-$(CH_2)_{12}$- TGA GGA TTT TGT AAG TTC CTT CA-3′

Biotinylated report probe DNA sequence:

RP-H1: 5′-AAT AGA CCT CTG GAT TGA AT-TEG-Biotin-3′

RP-H3: 5′-AGT GAC CTA AGG GTG GCA TA-TEG-Biotin-3′

RP-H9: 5′-GAT TCC AGC TTG ACC CCT TCT A-TEG-Biotin-3′

Random sequence:

RS-T: 5′-ACT GCT AGA GAT TAT CCA CAA TGA CTG CTT CAA CAG TGC CGC TAG-3′

2.2. Microfluidic chip design and fabrication

2.2.1. Microfluidic chip design

A microfluidic chip integrated a controllable micro-magnetic field and a heating region was designed (Figs. 1a, 1c, and 1d). The micro-magnetic field was used to generate the magnetic reaction area (Fig. 1b), and the heating region was used to control the hybridization temperature (Figs. 1a, 1c, and 1d). The integrated microfluidic chip (Figs. 1a and 1c) possessed three branch channels (width × height = 55 μm × 30 μm) which were used to detect multiple pathogens (H1N1、H3N2、H9N2) simultaneously. Two hand-made-miniature bars guiding magnetism (MBGM) were placed on the central position of channels with an angle of 120° (Figs. 1a and 1d), which were used to fix two magnets, and generate a mini-magnetic-field in the central position of channels (Zhang et al., 2013). Under the action of magnetic field, SMBs modified with different CP-DNAs were captured in each branch channel (Fig. 1b) for multiple pathogen (H1N1、H3N2、H9N2) detection (Fig. 1e).

2.2.2. Microfluidic chip fabrication

(1) ITO heating electrode fabrication. The ITO heating electrode was fabricated on ITO glass slide with the traditional soft-lithography. Briefly, AZ4620 photoresist was used to fabricate designed structure on ITO glass. Then ITO uncovered with photoresist was etched by the solution of 9 mol/L HCl and 1 mol/L $FeCl_3$. And finally, photoresist was washed off with anhydrous ethanol. The glass with ITO heating electrode was obtained (Figs. 1a, 1c and 1d). (2) PDMS microfluidic channel layer fabrication. The PDMS microfluidic channel layer was fabricated by soft lithography and rapid prototyped (Duffy et al., 1998). First, the 30-μm-thick AZ50XT master pattern on a silicon wafer was fabricated by soft lithography. Before PDMS casting, the silicon master was treated with trimethylchlorosilane vapor for 3 min to prevent the adhesion between PDMS and the silicon wafer. Then, two MBGMs were placed on the position beside the central channel with an angle of 120° (Figs. 1a and 1d), and the angle between the plane of the two MBGMs and that of the microfluidic channel was fixed at 90°. Finally, PDMS prepolymer (10:1 w/w RTV615A/RTV615B) degassed was poured onto the silicon master with thickness of 5–6 mm and baked at 75 °C for 3 h. Then the cured PDMS was peeled off from the master carefully after cooling to room temperature and punched with a blunt