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Magnetic nanoparticle-based immunoassay for rapid detection of influenza infections by using an integrated microfluidic system

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Abstract

Magnetic manganese ferrite ($MnFe_2O_4$) nanoparticles with approximately 100 nm in diameter were used to improve the performance of an immunoassay for detecting influenza infections. The synthesized nanoparticles were tested for long-term storage to confirm the stability of their thermal decomposition process. Then, an integrated microfluidic system was developed to perform the diagnosis process automatically, including virus purification and detection. To apply these nanoparticles for influenza diagnosis, a micromixer was optimized to reduce the dead volume within the microfluidic chip. Furthermore, the mixing index of the micromixer could achieve as high as 97% in 2 seconds. The optical signals showed that this nanoparticle-based immunoassay with dynamic mixing could successfully achieve a detection limit of influenza as low as 0.007 HAU. When compared with the 4.5-μm magnetic beads, the optical signals of the $MnFe_2O_4$ nanoparticles were twice as sensitive. Furthermore, five clinical specimens were tested to verify the usability of the developed system.

Key words: Influenza; Microfluidics; Nanoparticles; Surface modification

Introduction

Viral infectious diseases have been a serious threat to human health worldwide. The notorious acquired immunodeficiency syndrome instigated by the human immunodeficiency virus (HIV), for example, has caused the death of more than 10 million people for more than three decades.[1] Another serious disease is the severe acute respiratory syndrome (SARS), caused by SARS

Abbreviations: α-A-NP, anti-influenza A nucleoprotein; BSA, bovine serum albumin; C^+, normalized concentration; CCD, charge-coupled device; D^+, normalized location across the micromixer; DI, deionized; DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; EMVs, electromagnetic valves; FIA, fluorescence immunoassay; HAU, hemagglutin units; JCPDS, Joint Committee on Powder Diffraction Standards; LOC, lab-on-a-chip; LBL, layer-by-layer; mAbs, monoclonal antibodies; MDCK, Mardin–Darby canine kidney cells; MEMS, Micro-electro-mechanical systems; EDC, *N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide hydrochloride; One-way ANOVA, one-way analysis of variance; P123, poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer; PAA, poly(acrylic acid); PAH, polyallylamine hydrochloride; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PDMS, poly(dimethylsiloxane); PE, R-phycoerythrin; PEI, poly(ethyleneimine)); PMT, photo-multiplier tube; RB, round bottom; SARS, severe acute respiratory syndrome; TEM, transmission electron microscope; TPCK, type XIII l-1-tosylamide-2-phenylethyl chloromethyl ketone; UV, ultraviolet; XRD, X-ray diffractometer.

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coronavirus, which broke out in 2002.[2] More recently, in the winter of 2012, both norovirus and influenza virus caused widespread illness and infected millions of people. People infected with norovirus can develop acute gastroenteritis and become ill with influenza, causing sudden fever and whole-body achiness, as well as lung and heart diseases.[3] Furthermore, the influenza virus, which causes the flu, caused several historic pandemics; for instance, the Spanish Flu of 1918 claimed 27 million lives worldwide. The Hong Kong Flu in 1968 and the Bird Flu in 2004 also caused numerous deaths worldwide. The Swine Flu outbreak in June 2009 in Mexico caused serious concern because of the hydride genome of that virus.[4,5] Therefore, the capability to detect influenza infections rapidly and provide immediate and appropriate clinical treatment is an important need that needs to be addressed.

To diagnose influenza infection accurately, various types of diagnostic methods, such as viral culture, enzyme-linked immunosorbent assay (ELISA),[6] fluorescence immunoassay (FIA),[7] and molecular diagnosis using a real-time polymerase chain reaction (PCR),[8] have already been developed and applied in hospitals and laboratories. However, they are labor-intensive processes performed by well-trained personnel, and also require a substantial amount of bench-top equipment. Recently, with the development of micro-electro-mechanical systems (MEMS) technologies, an increasing number of miniature biomedical systems have demonstrated the potential for virus detection. For instance, a nano-cantilever beam operating as a mass detector was developed for the detection of viral particle[9]; this type of microsystem was further used for virus-specific antibodies to selectively detect pathogens.[10,11] However, sample pre-treatment and virus purification are still challenging to achieve in the cantilever beam sensing system.

In recent years, magnetic bead-based immunoassays have been integrated with microfluidic systems to realize effective micro-total-analysis system or lab-on-a-chip systems for pathogen detection.[12,13] Magnetic bead-based immunoassays were modified based on the sandwich-like immunoassays. Specific antibodies were coated on beads, which enabled the specific isolation and collection of target cells or viruses by using a magnetic force.[14] For instance, microfluidic systems demonstrating the capability of executing separations and detection of DNA fragments with small amounts of reagents in a single microfluidic device were achieved.[15,16] Rapid viral detection has also been demonstrated by several research groups.[17,18] For example, a microfluidic device with bead-based viral purification and on-chip reverse transcriptase PCR capabilities was applied to specifically detect dengue virus and enterovirus 71.[17] An integrated micro-flow cytometry using the bead-based immunoassay approach has demonstrated automatic detection of the dengue virus down to a concentration of 10^3 PFU/mL in 40 min.[18] Yet another integrated microfluidic system has achieved rapid detection in as little as 15 min which has been realized while using clinical specimens.[19,20] However, the magnetic beads used in previous studies are approximately 4.5 μm in diameter, which is much bigger than the influenza virus (approximately 100 nm in diameter). The incomparable size between beads and viruses can affect the detection signal and cause relatively high background noise. Using beads with smaller sizes and larger surface area may be beneficial for target detection.

With the advancement of nanotechnology, numerous nanomaterials have been developed for their unique properties. For instance, fullerenes and carbon nanotubes show high heat conductivity, electrical conductivity, and relative low chemical activity. They have even been used for medical studies associated with bacteria, viruses, and cancer cells.[21–23] Nanoparticles have also been developed for detecting avian flu in red blood cells[24] or alpha-fetoprotein in serum[25] because of their uniform distribution, long-term stability and bio-compatibility. Among them, magnetic nanoparticles have been applied in molecular imaging because of their outstanding magnetic-spin structures such that they could enhance magnetic resonance imaging.[26–28] Moreover, magnetic nanoparticles have advantages including multiplexing, reduced analysis time and selectivity control. Furthermore, with the development of microfluidic systems, magnetic nanoparticles have been used for immunoassay applications that involved a simple microfluidic chip. However, only immunoglobulin G was used as a test model and no disease detection was demonstrated.[29] For instance, superparamagnetic nanoparticles have been used to capture pathogenic microorganisms or nanometer-size particles, such as virions.[30,31] Immunoglobulin E detection in diagnosing allergies is another application of magnetic nanoparticles.[32] The high capture rate, throughput, and sample pre-concentration, as compared with the traditional assay, have been demonstrated.[33]

In this study, it is the first attempt to apply nanoparticles, by using an integrated microfluidic system that involves characterized micro-devices working with magnetic $MnFe_2O_4$ nanoparticles for influenza detection with a FIA. By using this approach, the detection limit of the diagnosis is expected to improve because of the large surface-to-volume ratio of nanoparticles. These well-produced stable magnetic $MnFe_2O_4$ nanoparticles are further combined with a layer-by-layer (LBL) surface modification process to reduce the background noise due to non-specific adhesion of nanoparticles. An integrated microfluidics system for detection of influenza infectious in 20 min has been demonstrated and may be promising for rapid diagnosis in the near future.

Materials and methods

Working principle of the diagnosis assay

Figure 1 shows a simplified diagram illustrating the diagnosis assay used in this study. The magnetic nanoparticles were surface-coated with anti-influenza A nucleoprotein (α-A-NP) monoclonal antibodies (mAbs), pre-loaded in a micromixer, and incubated with purified viral particle samples for 5 min (Figure 1, *A*). The purification of nanoparticle–virus complexes was realized by washing out non-specific interferences when an external magnetic field was applied for 2 min (Figure 1, *B*). After washing, the direct-conjugated R-phycoerythrin (PE) developing mAbs (α-A-NP–PE mAbs) was transported into the micromixer to incubate with the nanoparticle–virus complexes for 5 min (Figure 1, *C*). The non-binding interferences were washed away and the purification of the nanoparticle–virus-developing mAb complexes with the similar process.