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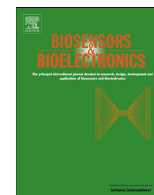

High sensitivity point-of-care device for direct virus diagnostics

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ABSTRACT

Influenza infections are associated with high morbidity and mortality, carry the risk of pandemics, and pose a considerable economic burden worldwide. To improve the management of the illness, it is essential with accurate and fast point-of-care diagnostic tools for use in the field or at the patient's bedside. Conventional diagnostic methods are time consuming, expensive and require specialized laboratory facilities. We present a highly sensitive, highly specific, and low cost platform to test for acute virus infections in less than 15 min, employing influenza A virus (H1N1) as an example of its usability. An all polymer microfluidic system with a functionalized conductive polymer (PEDOT-OH:TsO) microelectrode array was developed and exploited for label free and real time electrochemical detection of intact influenza A virus (H1N1) particles. DNA aptamers with affinity for influenza A virus (H1N1) were linked covalently to the conductive polymer microelectrodes in the microfluidic channel. Based on changes in the impedance when virions were captured by immobilized probes, we could detect clinically relevant concentrations of influenza A virus (H1N1) in saliva. This is a new, stable and very sensitive point-of-care platform for detection and diagnostics of intact virus particles.

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1. Introduction

Influenza infections are a major cause of morbidity and mortality, even though the vast majority of infections exhibits a self-limited, acute illness. In the United States alone, influenza infections pose a considerable economic burden with more than 200 000 hospitalizations and an average of 36 000 deceased of influenza-related causes every year (Thompson et al., 2003, 2004). Influenza A virus has the potential to generate global pandemics, illustrated by the influenza A virus (H1N1) 2009 pandemic, which affected more than 214 countries and was responsible for more than 18 000 deaths (Taubenberger and Kash, 2010).

Early identification of influenza as the cause of a respiratory illness is important for optimal patient management by allowing timely administration of antiviral treatment if needed. Current antiviral treatments for influenza have been shown to be effective in reducing severity, duration of illness and complications, but they should be started within 48 h after onset of symptoms (Nicholson et al., 2000; Treanor et al., 2000).

The reference standards for laboratory confirmation of influenza virus infection are reverse transcription-polymerase chain reaction (RT-PCR), direct antigen detection through fluorescent antibody staining, or viral culture. Viral culture requires 3–10 days, hence is not useful for patient management. Using the conventional immunofluorescence test to identify influenza antigens in clinical specimens produces results in 2–4 h, however this test depends heavily on laboratory expertise.

Molecular biological techniques for genome detection are increasingly being used in clinical settings, and they can identify subtypes of the influenza A virus within 3–8 h in an advanced laboratory setup. RT-PCR and other molecular detection methods are attractive, because they have high sensitivity and specificity. However, these methods do not indicate the viability of virus or on-going viral replication, and moreover, these methods are expensive, time consuming and require well equipped laboratories and trained personnel. RT-PCR is also associated with multiple potential technical errors, including failed extractions and problems with PCR inhibition (Bustin and Mueller, 2005).

To improve the management of influenza infections and virus infections in general, it is essential to provide accurate and timely point-of-care (POC) diagnostic tools for use in general practice or at the patient bedside. Currently, there are a number of POC tests available on the market to diagnose influenza A virus (H1N1) in clinical specimens, unfortunately these tests have demonstrated poor sensitivity and specificity (Babin et al., 2011; Bai et al., 2012; Gavin and Thomson, 2004), and inconsistent accuracy (Harper et al., 2009; Chartrand et al., 2012).

To compete with and outmatch the conventional techniques in virus diagnostics, the new generation of POC sensing devices must fulfil high standards and be producible at reasonable cost (Price and Kricka, 2007). Polymer based microfluidic systems meet the requirements of disposable devices, low sample consumption, cost

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efficiency, reliability, and fast response time, hence making the systems ideal for POC analysis. By selecting conducting polymers as electrode materials, the additional advantageous properties of inexpensive electrode fabrication and easy electrode functionalization can be achieved (Rozlosnik, 2009). The benefits of using conducting polymers as electrode material also include low cost compared to noble metals and high biocompatibility. Replacing metals with polymers does not only limit the cost on the raw material, but also allow for inexpensive mass production by modern inkjet printing methods (Loffredo et al., 2009; Mabrook et al., 2006) or agarose stamping (Hansen et al., 2007; Lind et al., 2012).

Seasonal influenza A virus spreads via respiratory secretions, and virus is shed in various specimens from the upper respiratory tract, such as nasal secretions or saliva specimens (Robinson et al., 2008). Nasal secretions are generally preferred due to high numbers of infected cells, which is important for conventional molecular diagnostic techniques. Saliva specimens are easily obtained but the virus concentration is lower (Robinson et al., 2008). However, utilizing a highly sensitive and highly specific POC device would neither require the presence of cells nor a high concentration of virus, consequently diagnosing influenza based on a saliva specimen would suddenly be doable.

We introduce a highly sensitive, highly specific, and very cheap method to test for acute virus infections in less than 15 min, using influenza A virus as an example. An all polymer microsystem with a functionalized conductive polymer (PEDOT-OH:TsO) microelectrode array was exploited for label free and real time electrochemical detection of intact influenza A virus (H1N1) particles. DNA aptamers with affinity for influenza A virus (H1N1) were linked covalently to the conductive polymer microelectrodes in the microfluidic channel. Based on changes in the electrical signal at the electrode surface when virus particles were captured by specific aptamers, we could detect clinically relevant concentrations of intact influenza A virus (H1N1). This is a new, stable and very sensitive platform for virus detection and diagnostics. In this system, intact virus is detected and quantified in its native configuration without destructing the particles, which is an advantage compared to RT-PCR.

2. Experimental procedures

2.1. All polymer microsystem

The all polymer microfluidic device has previously been described (Kiilerich-Pedersen et al., 2011). In brief, top and bottom pieces were fabricated from the cyclic olefin copolymer TOPAS 5013L (TOPAS Advanced Polymers, Germany; $Tg \approx 134\ ^{\circ}C$) by injection moulding (Victory 80/45 Tech, Engel, Germany). The top part contained access ports in standard luer lock size for fluid inlets, outlets and electrical connections. The second layer – microchannels and reservoirs – were defined in a 150 μm thick, pressure sensitive adhesive (PSA, ARcare 90106, Adhesive Research, USA) by laser ablation (Duo Laser CO_2 laser, Synrad Inc, USA).

Electrodes and electrical connection patches in the third layer were patterned in the conductive polymers, poly(3,4-ethylenedioxythiophene) doped with tosylate (PEDOT:TsO) and ((2,3-dihydrothieno[3,4-b][1,4]-dioxin-2-yl)methanol) (PEDOT-OH:TsO) using agarose stamping, described in detail by Dapra et al. (2013). In short, a stamp was prepared by casting a 10% w/w agarose gel into a mould with a relief of the electrode design etched in silicon. After solidification the stamp was soaked in the etching solution and applied manually to the PEDOT:TsO/PEDOT-OH:TsO bilayer. For etching a 1–1.5% w/v solution of sodium hypochlorite in water containing 0.1% v/v of the surfactant TritonX100 was used. The manual stamping time varied between 45 and 60 s. Although the interdigitated microelectrodes were designed with a width of 20 μm and with the same spacing between single electrodes, due to the uncontrolled diffusion of sodium hypochlorite into the polymer, the width of electrodes varied between 8.1 μm and 14.3 μm with a mean of 10.6 μm, changing the sensitivity of the device. After stamping, over-oxidized PEDOT was removed by washing with water. The conducting polymer layers were re-doped by immersion in 4% w/v $Fe^{(III)}$tosylate solution for a few seconds.

The final chip was assembled by thermal assisted bonding at 75 °C with 500 N force applied for 5 min in an in house build, LabView (National Instruments, USA) controlled press. In an assembled device, the volume of the microchannel was 100 μL.

2.2. Oligonucleotides

An oligonucleotide with the following sequence was used in this study: 5′AAT TAA CCC TCA CTA AAG GGC TGA GTC TCA AAA CCG CAA TAC ACT GGT TGT ATG GTC GAA TAA GTT AA-3′ (A22 presented by Jeon et al. (2004)) (DNA Technology A/S, Denmark). The molecule was functionalized with an 5′−amino modified C_6 linker.

2.3. Virus

Influenza A virus (H1N1, strain A/PR/8/34, ATCC Cat. No. VR-1469; LOT:59252244) was aliquoted and stored at −80 °C.

2.4. Electrode functionalization

Succinic acid was grafted onto surface hydroxymethyl groups with 0.1 M MES buffer (2-(*N* -morpholino)ethanesulfonic acid) at pH 4.0 supplemented with 50 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 50 mM succinic anhydride for 20 min at room temperature. Following, the surface carboxylic acid groups were activated with 50 mM EDC and 40 mM NHS (*N* -hydroxy succinimide) in MES buffer (0.1 M, pH=4.0) for 5 min. The microchannel was washed with MES buffer (0.1 M, pH=4.0), and then the activated acid intermediate reacted with the 5′amino group of the aptamer at a concentration of 100 nM in water to form a stable amide bond. Reaction was allowed to proceed over night and then the microchannel was rinsed with PBS, and kept in this solution until the virus measurement. The electrochemical impedance spectra – during the immobilization process – are shown in the Supplementary information.

2.5. Aptamers

Aptamers are nucleic acid ligands, generated in vitro from a randomized pool of molecules by affinity and amplification processes (Bunka and Stockley, 2006). DNA aptamers were employed in this study due to the advantageous properties over antibodies, and because aptamers are now recognized as substitutes for antibodies on the diagnostic front (Gopinath, 2007). Aptamers are generated with a high degree of control, giving a more consistent quality than antibodies, which are produced in a biological system, with a batch to batch variation and much shorter shelf life.

The applied ssDNA aptamer A22 binds directly to the globular region of the influenza glycoprotein hemagglutinin (HA) (HA-(91–261)) (Jeon et al., 2004) of intact influenza A virus (H1N1). Unlike most monoclonal antibodies, the aptamer A22 binds highly specific to its target, and can discriminate between virus strains within the influenza subtypes, based on differences in the amino acid residues (Gopinath, 2007).

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