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Multiplexed efficient on-chip sample preparation and sensitive amplification-free detection of Ebola virus

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ABSTRACT

An automated microfluidic sample preparation multiplexer (SPM) has been developed and evaluated for Ebola virus detection. Metered air bubbles controlled by microvalves are used to improve bead-solution mixing thereby enhancing the hybridization of the target Ebola virus RNA with capture probes bound to the beads. The method uses thermally stable 4-formyl benzamide functionalized (4FB) magnetic beads rather than streptavidin coated beads with a high density of capture probes to improve the target capture efficiency. Exploiting an on-chip concentration protocol in the SPM and the single molecule detection capability of the antiresonant reflecting optical waveguide (ARROW) biosensor chip, a detection limit of 0.021 pfu/mL for clinical samples is achieved without target amplification. This RNA target capture efficiency is two orders of magnitude higher than previous results using streptavidin beads and the limit of detection (LOD) improves 10×. The wide dynamic range of this technique covers the whole clinically applicable concentration range. In addition, the current sample preparation time is ~1 h which is eight times faster than previous work. This multiplexed, miniaturized sample preparation microdevice establishes a key technology that intended to develop next generation point-of-care (POC) detection system.

1. Introduction

Ebola is one of several known hemorrhagic fever viruses that causes an acute and serious illness in humans and other mammals. It is highly contagious and without urgent treatment leads to damage of blood vessels, organ dysfunction, and death. The recent outbreak of Ebola began in 2013 in West Africa and spread to nearly 10 countries and resulted in significant loss of life (Nouvellet et al., 2015). Since an Ebola vaccine and antiviral therapy are still not available, a rapid and simple point-of-care (POC) diagnostic test would help to control its spread by interrupting the transmission cycle. Although polymerase chain reaction (PCR) (Muyzer et al., 1993) and enzyme linked immunoassays (ELISA) (Engvall and Perlmann, 1971) are now considered the gold standard for pathogen detection, they require bulky equipment, specialized laboratories, carefully curated and refrigerated reagents, and well-trained personnel which makes them problematic for routine use in low resource locations. The transportation of samples to specialized laboratories can also delay testing which increases the chances of viral transmission. Amplification-free nucleic acid detection, on the other hand, can provide a rapid and simple diagnosis test at POC which does not require specialized training and facilities. Rapid detection at local clinics would help to interrupt virus transmission by identifying positive case and unambiguously excluding the unaffected.

A number of amplification-free techniques such as plasmonic sensing (Jackman et al., 2015; Stockman, 2015), electrochemical detection (Gau et al., 2005; Henihan et al., 2016), and advanced microscopy (Klamp et al., 2013; Tao et al., 2015) have been introduced to achieve sensitive detection of nucleic acid targets diagnostic of pathogens. Plasmonic sensing, relying on the spectral shift of plasmonic resonances, requires complicated fabrication techniques of noble metal nanostructures, which makes it non-ideal for low-cost and disposable devices. Electrochemical detection does not require expensive and slow patterning processes. However, the detection sensitivity is poor without target amplification. Confocal or wide-field microscopes are very sensitive but microscopes are bulky and difficult to operate at POC and require expert image interpretation. Our own work developed a viral detection technique based on an antiresonant reflecting optical

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waveguide (ARROW) which creates femtoliter excitation volumes on a chip to effectively excite and detect individual labeled target DNA or RNA molecules (Ozcelik et al., 2015; Schmidt and Hawkins, 2011). The fluorescent burst signal is recorded as reagents flow through the liquid-core. This optofluidic platform has demonstrated single molecule detection capability in the laboratory but the extension of this powerful technique for routine use in the field requires coupling with automated sample preparation (Cai et al., 2015; Liu et al., 2015; Schmidt and Hawkins, 2016).

Microfluidics with its advantages of low volume consumption, flexibility, disposability, and low costs, is a powerful tool for addressing POC sample preparation challenges (Elvira et al., 2013; Sackmann et al., 2014; Tan et al., 2015; Thompson et al., 2015). Because the channel and reactor sizes are at the micrometer scale, multiple assays can be performed in a small area in parallel. However, due to the low specific Reynolds number of liquids in microfluidic channels and reservoirs, the mixing of regents is limited due to the absence of turbulent or convective mixing (Lee et al., 2011; Wang et al., 2014). Equilibration of different phases such as solid beads and liquid is even more difficult in microfluidic devices. Several techniques have been introduced to enhance mixing in microfluidic systems, but most require additional complex devices and processes such as high DC voltage (Fang et al., 2015), microelectromechanical systems (Frommelt et al., 2008; Suzuki et al., 2004) and rotors (Jackson et al., 2016; Strohmeier et al., 2015). We thus sought to develop a simple and efficient technique to improve microfluidic mixing that does not include complex fabrication or devices.

Recently, the hybrid optofluidic integration of microfluidic sample preparation and optical sensing has come to the fore (Parks et al., 2014; Testa et al., 2014). This has led to the successful demonstration of amplification-free detection of Ebola nucleic acids on an ARROW chip, following partial sample preparation on a microfluidic chip (Cai et al., 2015). While a low limit of detection and a large dynamic range were demonstrated, the performance of the system did not reach the

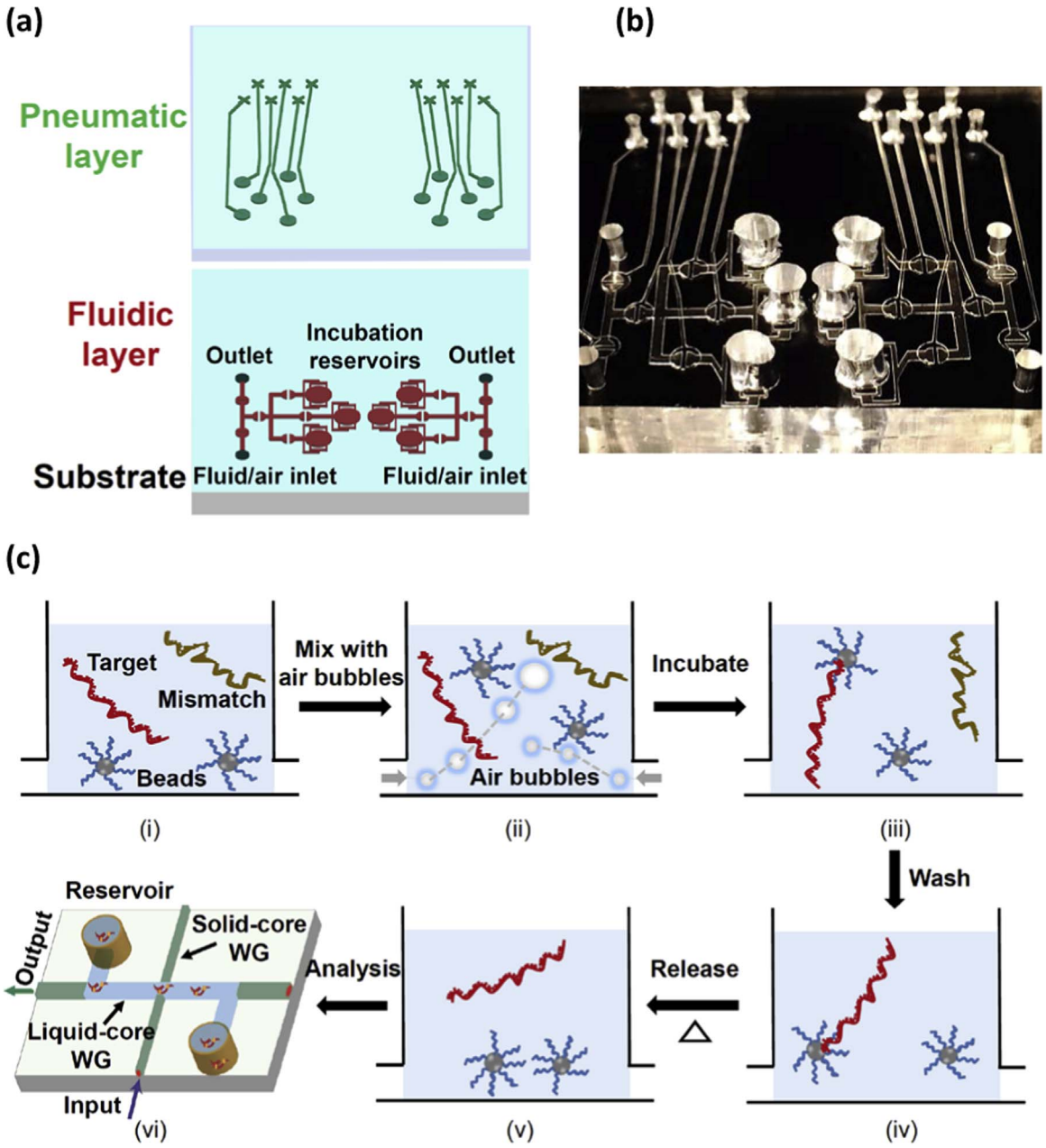

Fig. 1. (a) Design of the sample preparation multiplexer (SPM) with six incubation reservoirs for target preparation. Reagents and metered air bubbles are introduced into the incubation reservoirs by microvalve pumps. (b) Photograph of the SPM. (c) Schematic of the solid-phase extraction process and assay.