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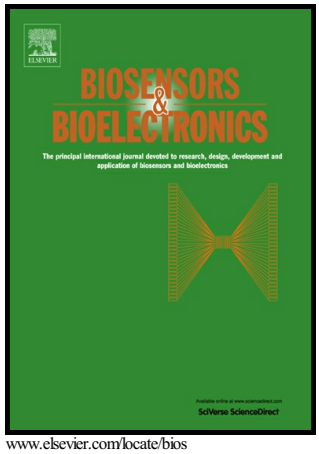

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A bead-based immunofluorescence-assay on a microfluidic dielectrophoresis platform for rapid dengue virus detection

Edwar Iswardy[a,b], Tien-Chun Tsai[a], I-Fang Cheng[c], Tzu-Chuan Ho[d,e], Guey Chuen Perng[d,e,f*], Hsien-Chang Chang[a,g*]

[a]Institute of Biomedical Engineering, National Cheng Kung University, Tainan, 70101, Taiwan

[b]Department of Physics, Syiah Kuala University, Banda Aceh, 23111, Indonesia

[c]National Nano Device Laboratories, National Applied Research Laboratories, Tainan, 70101, Taiwan

[d]Department of Microbiology and Immunology, National Cheng Kung University, Tainan, 70101, Taiwan

[e]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, 70101, Taiwan

[f]Center of Infectious Disease and Signaling Research, National Cheng Kung University, Tainan, 70101, Taiwan

[g]Medical Device Innovation Center, National Cheng Kung University, Tainan, 70101, Taiwan

[*]Corresponding author. Present address: National Cheng Kung University, No. 1 University Rd, Tainan City 70101, Taiwan. gperng@mail.ncku.edu.tw

[*]Corresponding author. Present address: National Cheng Kung University, No. 1 University Rd, Tainan City 70101, Taiwan. hcchang@mail.ncku.edu.tw

Abstract

The proof of concept of utilizing a microfluidic dielectrophoresis (DEP) chip was conducted to rapidly detect a dengue virus (DENV) *in vitro* based on the fluorescence immunosensing. The mechanism of detection was that the DEP force was employed to capture the modified beads (mouse anti-flavivirus monoclonal antibody-coated beads) in the microfluidic chip and the DENV modified with fluorescence label, as the detection target, can be then captured on the modified beads by immunoreaction. The fluorescent signal was then obtained through fluorescence microscopy, and then quantified by ImageJ freeware. The platform can accelerate an immuno-reaction time, in which the on-chip detection time was 5 min, and demonstrating an ability for DENV detection as low as 10^4 PFU/mL. Furthermore, the required volume of DENV samples dramatically reduced, from the commonly used ~50 µL to ~15 µL, and the chip was reusable (>50x). Overall, this platform provides a rapid detection (5 min) of the DENV with a low sample volume, compared to conventional methods. This proof of concept with regard to a microfluidic dielectrophoresis chip thus shows the potential of immunofluorescence based-assay applications to meet diagnostic needs.

Keyword: dengue virus, immunofluorescence-assay, dielectrophoresis, microfluidics, biosensor.

1. Introduction

Dengue is an emerging viral infection in tropical and sub-tropical regions, transmitted primarily by the *Aedes aegypti* mosquitoes, with four dengue virus serotypes recognized to date. Dengue epidemics are growing worldwide, with an annual infection incidence of approximately 50-100 million (WHO, 2011), and an estimated 40% of the world's inhabitants are at risk of dengue virus (DENV) infection (Bäck and Lundkvist, 2013). Climate change and environmental disturbances (Brady et al., 2014; Liebman et al., 2012; Messina et al., 2014), globalization and international travel (Gubler, 2011; Ratnam et al., 2013; Weaver, 2013), and persistent DENV infections (Chastel, 2012) all play important roles in the spread of this disease.

Clinical manifestations of DENV infection range from asymptomatic to dengue fever and dengue hemorrhagic fever/dengue shock syndrome (WHO, 2011). However, it is estimated that three quarters of the 390 million DENV infections that occur in the world each year are typically asymptomatic or mildly symptomatic (Bhatt et al., 2013), and frequently undetectable (Peeling et al., 2010). Studies on the development of dengue show that people with asymptomatic DENV infection could be a potentially persistent carrier of dengue epidemic transmission (Perng, 2015). The virus can be transmitted to humans via mosquitoes bites, blood transfusions (Linnen et al., 2008; Stramer et al., 2012), stem cells engraftment, bone marrow and organ transplantations (Franco-Paredes et al., 2010; Greenwald et al., 2012; Gupta et al., 2016), and also by pregnancy (Paixão et al., 2016; Singla et al., 2015). Asymptomatic dengue virus infections are more efficient in spreading DENV than symptomatic infections, because people with these infections are likely to be exposed to more mosquitoes during their undisturbed daily routines, and this phenomenon is crucial phase in dengue epidemiology (Duong et al., 2015).

Since the clinical symptoms of dengue are similar to many common febrile illnesses, it is challenging to diagnosis it correctly. There are many commercially available rapid diagnostic

tests, mainly based on the soluble dengue viral antigen (nonstructural protein 1, NS1) and specific antibody to DENV. The co-circulation of other common mosquito-borne viral diseases in the same temperal zones makes the read out and interpretation for specific DENV infections far more difficult than initially thought. In addition, although a much more specific method, viral RNA detection, is available, the long detection time with this is a limiting factor. In terms of accuracy, viral particle detection would be more effective if applied for DENV infection diagnosis, however, for reasons of convenience serological detection is a better option (Peeling et al., 2010). As such, an alternative test for detecting DENV particles that is rapid, accurate and easy to handle is needed. As such, in recent years there has been much research on biosensor-based DENV particle detection. The electrochemical impedance spectroscopy method was used to detect viral particle analytes in spiked serum with a limit of detection (LoD) of 1 PFU/mL and assay time of 50 min (Cheng et al., 2012), while another study reported that the LoD was 0.23 PFU/mL for dengue type 2 virus and 0.71 PFU/mL for dengue type 3 virus, both within 40 min (Peh and Li, 2013). However, electrochemical techniques have some limitations, such as electrochemical interference, weak long-term stability, and complicated electron-transfer pathways (Wang et al., 2008). Nevertheless, the evidence on biosensor-based dengue virus detection is very promising with regard to sensitivity and specificity, as well as its greater speed, lower requirement for samples and ease of miniaturization.

Amongst the various biosensor-based methods that have been developed, dielectrophoresis (DEP) is a popular approach which allows the manipulation of bioparticles based on their size, shape, and dielectric characteristics (Cheng et al., 2014). DEP biosensors have some distinct advantages for bioparticle manipulation, including separating, concentrating, directing, and capturing (Qian et al., 2014), and can be integrated with other approaches such as optical,

electrochemical and microfluidic techniques. To date, our laboratory has intensively developed and established microfluidic and DEP methods for a range of applications, such as DNA and protein detection, bacteria detection, bioparticles and blood cell separation, antibiotic susceptibility testing, cancer cell discrimination and bead-based immunoassay (Cheng et al., 2009; Chung et al., 2011; Liao et al., 2013).

In this work, we have developed a proof of concept diagnostic platform using a DEP method integrated with a microfluidic chip to be applied for DENV particle detection. This platform aims specifically to detect the existence of DENV particles in analytes based on fluorescence signals from the interaction between 4G2-coated beads and a fluorescent probe-labeled DENV. This system is as well designed to address the drawbacks of conventional methods by shortening the time needed, and reducing both the amount of sample required and the operating costs. To reduce the detection time, a bead-based assay method was used to increase the reaction time due to the high surface-to-volume-ratio of beads, while a non-uniform electric field was applied on the chip to generate the dielectrophoretic force needed to guide and capture the samples based on their dielectric properties.

2. Material and methods

2.1. Chip design and fabrication

A three-dimensional dielectrophoresis microfluidic chip was made of Au/Ti/glass slide using a standard photolithography process (Cheng et al., 2012), with some modifications. The production of microelectrodes is the first step in chip fabrication, followed by the formation of microchannel. Furthermore, chip bonding was carried out to shape a three-dimensional

microfluidic chip. The entire chip fabrication protocol is shown in the Supplementary Information (Fig. S1).

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2.2. Anti-flavivirus monoclonal antibody (4G2) immobilization on silica beads

Silica beads (1 µm) with the carboxylic functional group (COOH) were prepared by suspending 1 g solid silica beads in 1 mL deionized water. A number concentration of 10^8 beads/mL of silica bead suspension was used to immobilize antibodies on the surfaces. The antibody immobilization protocol followed the product data sheet of the Polylink protein coupling kit for COOH Microspheres (Bangs Laboratories, Inc.), consisting of coupling buffer (50 mM MES, pH 5.2; 0.05% Proclin®300), washing/storage buffer (10 mM Tris, pH 8.0; 0.05% Bovine Serum Albumin; 0.05% Proclin®300), EDC (1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide), as shown in the Supplementary Information. The EDC functions to crosslink carboxyl to the amine group of protein. NHS was included in the EDC coupling protocols to improve conjugation efficiency or reaction stability with regard to the primary amine group at physiologic pH. Mouse anti-flavivirus monoclonal antibody (4G2) was used in this study as the capture antibody that would be immobilized on the beads. The immobilization procedure is shown in the Supplementary Information (Fig. S2), and flow cytometric analysis (BD Flow Accurri C6 Plus and the Karuza software) was employed to examine the immobilization of mouse anti-flavivirus (4G2) on silica-COOH beads. Flow cytometric analysis was conducted by using the fluorescence-activated cell sorting (FACS) method, as this is a powerful tool to sort and investigate chemical or biological particles based on their fluorescent properties (Ambriz-Aviña, et al., 2014).

2.3. Dengue virus labeling with fluorescence probe

The dengue type 2 virus was kindly provided by Dr. Perng's lab with the approximate diameter of 40-50 nm which is in line with the prior reports (Hurrelbrink and McMinn, 2003; Kuhn et al., 2002). The dengue type 2 virus stock was stored in Dulbecco's Modified Eagle's Medium (DMEM comprising 2% FBS, 1% Sodium Pyruvate, 1% Sodium bicarbonate, and 1% antibiotic) at -80^{o}C and then melted at 4^{o}C for fluorescent probe labeling. 1 µL anti-polyclonal envelope protein of dengue virus (1 mg/mL, GeneTex) was then added to the dengue virus stock in the ratio 1:1000. This mixture was incubated at 4^{o}C for 1 h and interspersed by mixing gently end-to-end using a pipette every 15 min to allow optimal conjugation. After that, 2 µL donkey anti-rabbit IgG-AF488 (2 mg/mL, Invitrogen) was added to the mixture in a ratio of 1:5000. This mixture was also incubated at 4^{o}C for 1 h and interspersed by mixing end-to-end gently every 15 min to allow optimal binding. DENV conjugated anti-DENV envelope protein antibody conjugated donkey anti-rabbit IgG-AF 488 was finally obtained in DMEM storage buffer with the conductivity, σ, of 15 mS/cm. FACS analysis was also performed to analyze the conjugation of donkey anti-rabbit IgG-AF 488 fluorescent probe labeled on dengue virus conjugated anti-envelope protein antibody. The entire labeling protocol is presented in the Supplementary Information (Fig. S4).

2.4. Chip pretreatment and sample preparation

Chip pretreatment was employed by injecting and coating the microchannel with 1% Bovine Serum Albumin (BSA, Sigma Aldrich) for 30 min to avoid the particles sticking on the channel walls or electrode surface (Cheng et al., 2012). This step has to be applied shortly before the on-

chip experiment. For the next step the samples need to undergo pretreatment before being applied on the chip. The 4G2-coated silica beads were stored in Tris buffer (10 mM Tris, pH 8.0; 0.05% Bovine Serum Albumin; 0.05% Proclin®300) at 4°C. The Tris buffer was changed to DMEM buffer with the conductivity 1 mS/cm. Centrifugation (EBA 21) was carried out at 6000 rpm for 5 min to pellet the 4G2-coated beads. After removing the supernatant, the 4G2-coated silica bead pellets were then resuspend in DMEM. Gentle pipetting was used to prevent aggregation. Another sample that was a DENV mixture stock (DENV conjugated with anti-DENV envelope protein antibody-donkey anti-rabbit IgG-AF488) was stored in DMEM (2% FBS, 1% Sodium Pyruvate, 1% Sodium Bicarbonate, and 1% antibiotic) with the conductivity, σ, of 15 mS/cm. By diluting with DI water in the ratio 1:18 (DMEM:DI water) the DENV mixture was obtained in DMEM buffer with the conductivity set at 1 mS/cm. Varying concentration of the samples were obtained by serial dilution.

2.5. Experimental configuration

The equipment used in the experiment was configured as shown in Fig. 1A. A syringe pump (KD-Scientific) was used to inject the samples via the inlet port. An arbitrary waveform generator (FLUKE 284) was used to supply the AC voltage to the chip. For image display and recording a CCD camera (QIMAGING-RTV) was used along with an inverted fluorescent microscope (OLYMPUS IX70). The *in vivo* detection model for dengue virus prepared in previous steps was then applied on the chip platform. The 4G2 coated-beads were first injected to be captured on the capturing electrode, with the amount controlled by using the guiding electrode. After obtaining an appropriate number, the injection of the 4G2-coated beads and application of the guiding electrode were terminated. After this, the dengue virus labeled

fluorescent probe were immediately injected to interact with the 4G2-coated beads, with the resulting fluorescent signals being the detection results. This detection mechanism, comprising sample capturing and guiding, is shown in Fig. 1B and Fig. 1C.

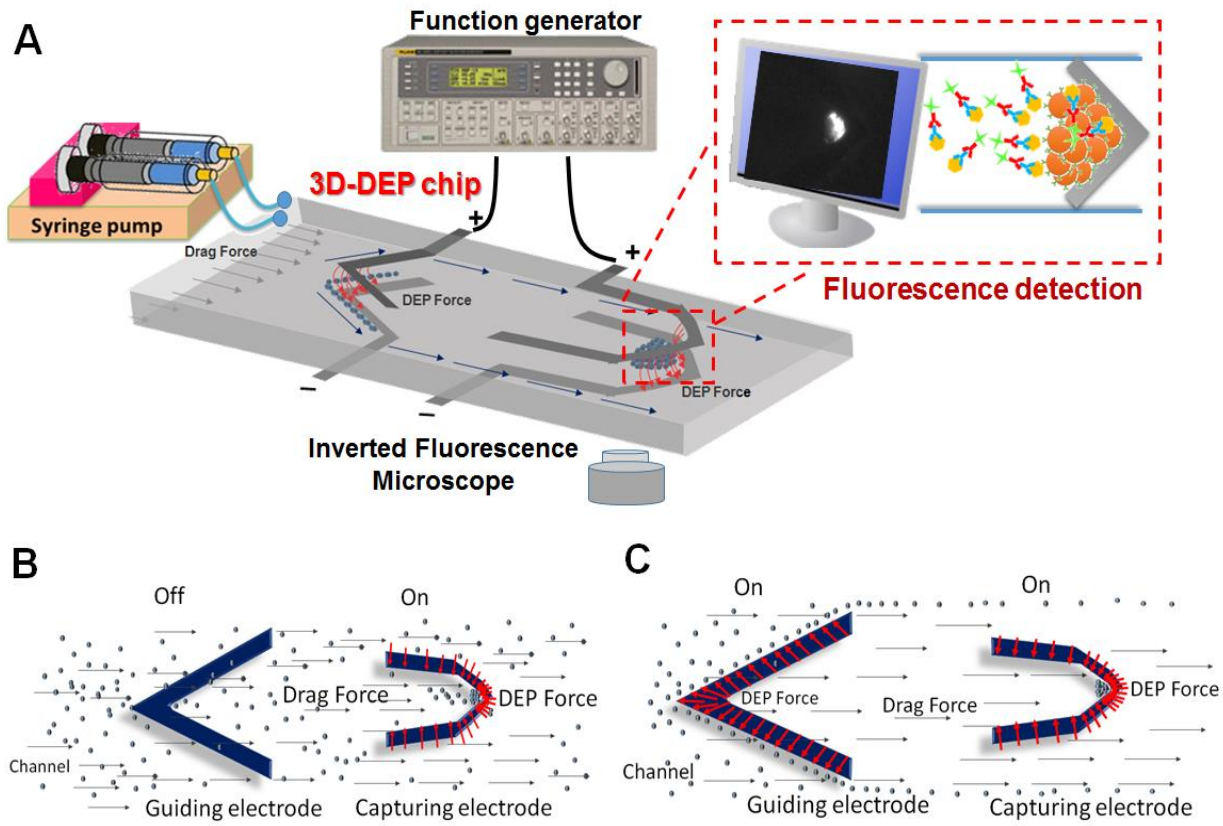

Fig. 1. (A) Experimental set-up of the system for dengue virus detection. Schematic of the mechanism of detection including sample (B) capturing and (C) capturing and guiding sequentially. The tip-to-tip width, the width and the angle of the guiding electrodes are 90 μm, 30 μm and 20^{o} respectively and of the capturing electrode are 25 μm, 20 μm and 45^{o} respectively. The red arrows on the electrodes mean that the AC voltage had been applied.

The DEP force on a spherical particle induced by a non-uniform electrical field is expressed by $F_{DEP} = 2\pi\varepsilon_m\, r^3 Re[f_{CM}(\omega)]\nabla|E|^2$ (Jones, 1995; Morgan and Green, 2003). This formula describe that the DEP force depends on the permittivity of the suspending medium (ε_m), the radius of particles (r), the gradient of electric field squared ($\nabla|E|^2$) and the real part of the frequency dependent Clausius-Mossotti (CM) factor ($Re[f_{CM}(\omega)]$). The $f_{CM}(\omega)$ can be defined by $(\varepsilon_p^* - \varepsilon_m^*)/(\varepsilon_p^* - 2\varepsilon_m^*)$ where ε_p^* and ε_m^* represent the frequency dependent complex permittivity of the particle and the medium, respectively. The complex permittivity is given as $\varepsilon_p^* = \varepsilon_p - i(\sigma_p/\omega)$ and $\varepsilon_m^* = \varepsilon_m - i(\sigma_m/\omega)$ where σ_p and σ_m are the conductivity of the particle and the medium, respectively. These permittivity and conductivity will affect the polarization of the particle. When the particles is more polarizable than that of the medium, the DEP force will push the particle towards the stronger electric field gradient (positive DEP). Conversely, when the particles are less polarizable than that of the medium, the particles will travel towards the weak electric field gradient (negative DEP). Since the polarizabilities of the particle and the medium are frequency-dependent, thus the CM factor can be set by tuning the applied frequency (ω) and will influence the direction of the DEP force on the particle. Particles in continuous flow will also experience hydrodynamic force following Stokes's Law $F_{drag} = 6\pi\eta r v$ where η is the viscosity of the medium, *r* is the radius of the particle, and *v* is the speed of fluid flow and gravitation force, g. These forces were employed for sample capturing and guiding during the detection process. The balance of a drag force and a DEP force ($F_{DEP} = F_{drag}$) will allow capturing the particles in three-dimensional microfluidic DEP chip with the V-shaped capturing electrodes (Cheng, 2012). The guiding electrode arc was designed to be wider than the V-shaped capturing electrodes arc to allow the samples to flow, and thus avoid the funnel

capturing electrodes, as well as enabling control of the amount of sample captured on the V-shaped electrodes.

2.6. Quantification of fluorescence intensity

The fluorescent images obtained from fluorescence microscope are qualitative data. The ImageJ freeware was utilized to quantify the fluorescent images to assess the relative fluorescence intensity, following the protocol shown in the Supplementary Information (Fig. S6).

3. Results and discussion

3.1. Binding affinity evaluation of mouse anti-flavivirus (4G2) antibody immobilized on silica beads

FACS analysis was used to assess 4G2 immobilization on the surfaces of the silica beads. The donkey anti-mouse IgG-AF 488-conjugated beads were utilized as the negative control, while 4G2-coated beads with conjugated donkey anti-mouse IgG-AF 488 were used to demonstrate the validity of the model designed for DENVcapture using mouse anti-flavivirus (4G2) monoclonal antibody. As shown in the Supplementary Information (Fig. S3), the negative control results do not show fluorescence signals, which means that when mouse anti-flavivirus antibody is not used then no binding occurs between the silica beads and donkey anti-mouse IgG AF 488. In contrast, 4G2-coated beads and donkey anti-mouse IgG-AF 488 show the desired conjugation, as indicated by the significant signals at the 10^4 peak of fluorescence intensity. This

means that 4G2 has been successfully immobilized on the surface of silica beads, has high binding affinity with DENV, and so can serve as a capture antibody for DENV.

3.2. Binding affinity evaluation of DENV labelling with AF 488 fluorescence probe.

DENV type 2 was labeled with fluorescence probe AF 488 following the protocol as explained in the Supplementary Information (Fig. S4). FACS analysis was performed to assess the binding affinity between DENV and the AF 488 fluorescence probe tagged on donkey anti-rabbit IgG conjugated anti-DENV envelope protein as the DENV detection model (Fig. 2C). As the negative control, two groups of interaction were also carried out. The first group was 4G2-coated beads labeled directly to AF 488 (Fig. 2A), and the second group is 4G2-coated beads conjugated to anti-DENV envelope protein antibody conjugated donkey anti-rabbit IgG AF 488 (Fig. 2B). As shown in Fig. 2C, the FACS analysis revealed that the detection model showed a significant fluorescence signal, which means AF 488 labeled secondary antibody donkey anti-rabbit IgG conjugated anti-DENV envelope protein antibody is successfully conjugated to DENV, as represented by the fluorescent peak shift located at 10^4-10^5 compared to the control. This means that the whole detection model is workable, and can be applied for the proposed platform assay.

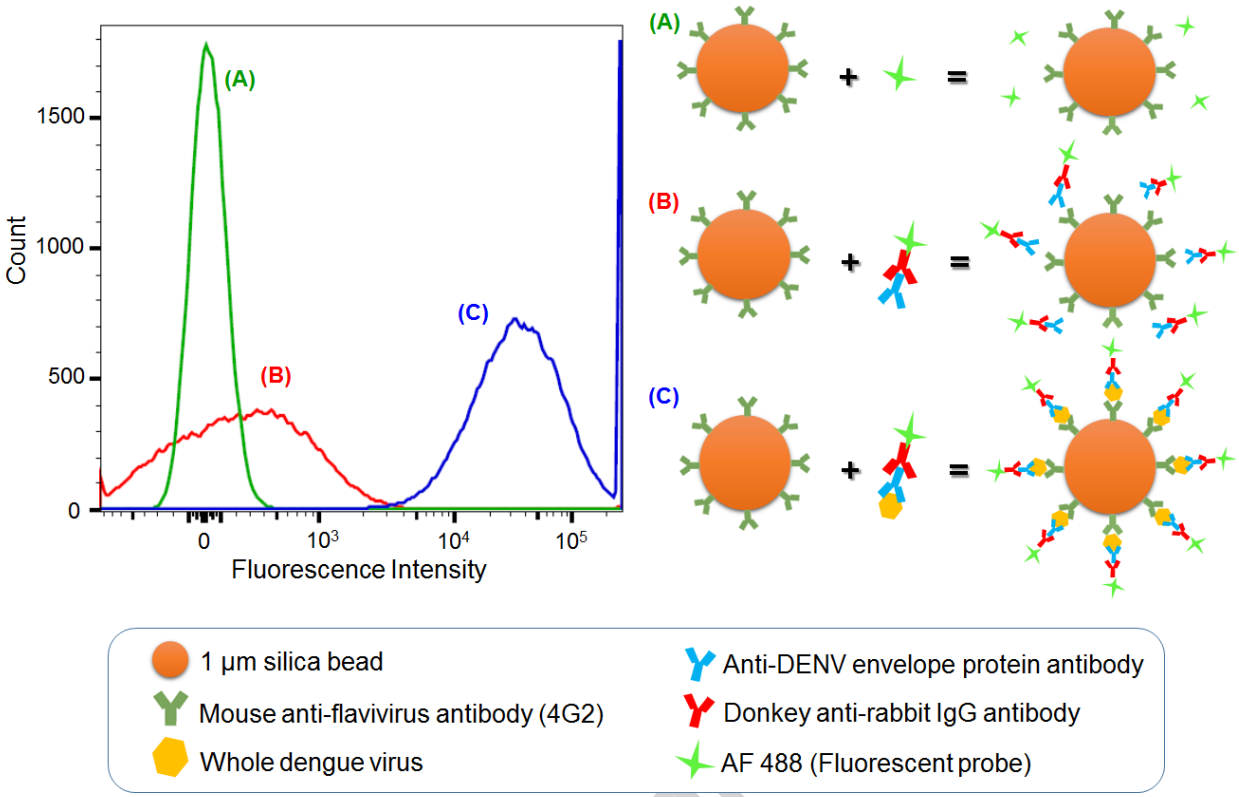

Fig. 2. FACS analysis of fluorescence probe AF 488 labeling on DENV and negative controls. (A) Negative control of 4G2-coated beads labeled directly to AF 488. (B) Negative control of 4G2-coated beads conjugated to anti-DENV envelope protein conjugated donkey anti-rabbit IgG AF 488. (C) Fluorescence probe labeling was applied using AF 488 tagged on secondary antibody donkey anti-rabbit IgG conjugated to anti-DENV envelope protein conjugated to DENV, and this showed fluorescence peak intensity at 10^4-10^5.

3.3. Chip performance for guiding and capturing samples

The results show the operating parameters of the three-dimensional microfluidic DEP chip to perform guiding, capturing and detecting function. 1 μm bare silica beads in deionized water with conductivity, σ, of 1 μS/cm was used to show guiding (Fig. 3A) and capturing (Fig.

3B) performance. It is found that the guiding and capturing functions could be effectively performed at AC voltage of 15 $V_{pp,}$ frequency of 1 MHz and flow rate of 0.3 µL/min. In this condition, the silica beads experienced negative dielectrophoresis (nDEP) force, and thus they are repelled from a strong electric field gradient at the tip of the electrode. On the other side, a hydrodynamic force (F_{drag}) was subjected on the beads. A balanced force between nDEP and F_{drag} is required to capture beads at the cusp region of the chip (Cheng et al., 2012). For the guiding function, the electrode will direct the bead stream to avert the capturing electrode, and hence the amount of samples captured by the V-shaped capturing electrodes can be well controlled. The guiding and capturing functions were then applied to 4G2-coated beads, as shown in Fig. 3C. The 4G2-coated beads was started to be captured by applying a voltage on the capture electrode and the number of 4G2-coated beads was started to be controlled by applying a voltage on the guiding electrode after 30 s capturing. Based on the 4G2-coated bead-captured area, it can be seen that the number of 4G2-coated beads was controlled at 30 to 60 s, and the number of captured 4G2-coated beads stabilized within 60 s of the capturing time with the volume in average 1966 $\mu m^3 \pm 20.3\%$. Controlling a certain amount of the 4G2-coated beads will allow optimal interaction between the 4G2-coated beads and the DENV mixture, and thus will increase the captured fluorescence intensity. The raw data for the 4G2-coated bead guiding and capturing is shown in the Supplementary Information, Fig. S5 and Table S1.

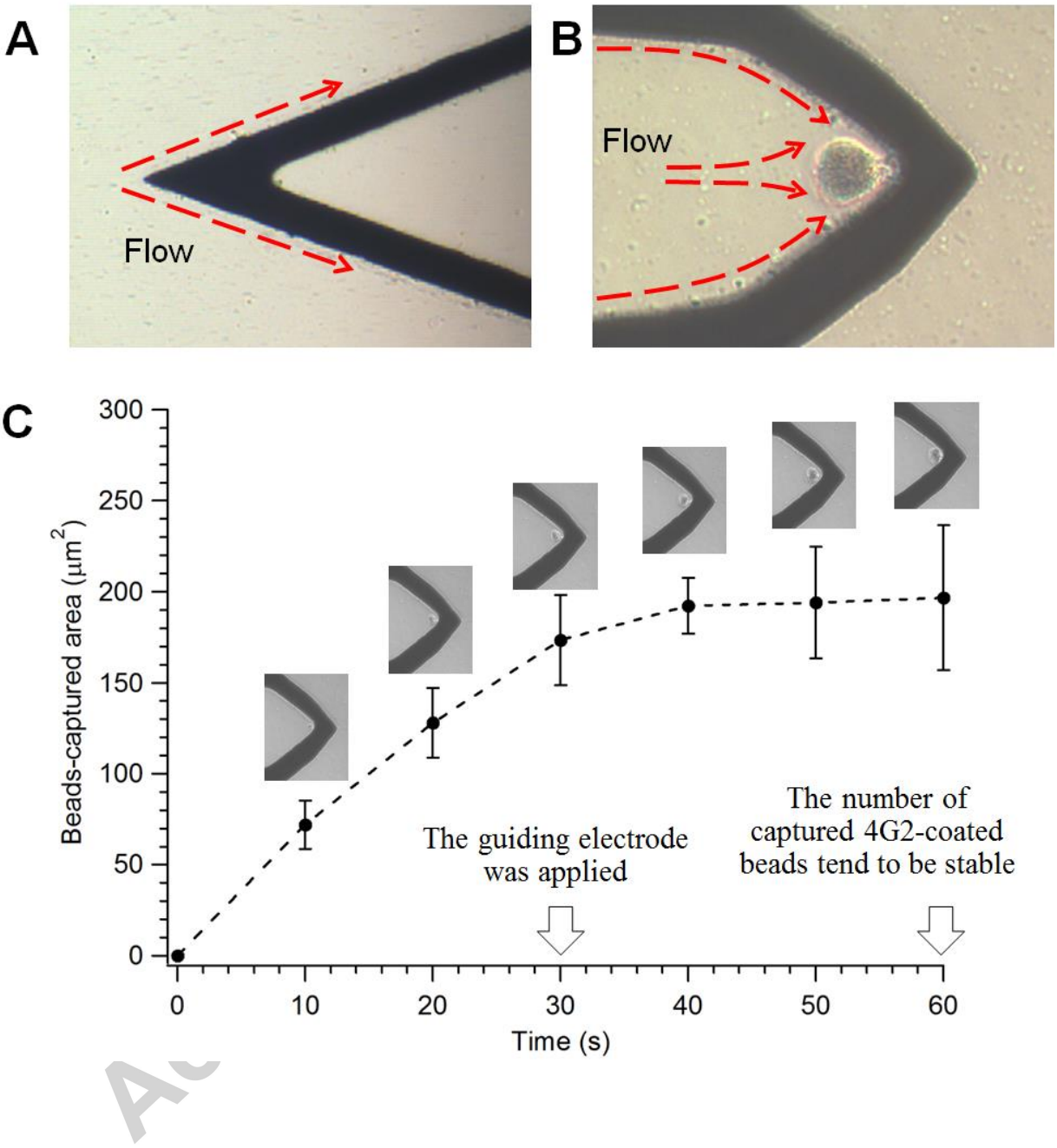

Fig. 3. (A) Guiding and (B) capturing functions of silica beads, in deionized water with the conductivity of 1 μS/cm and the operating parameters adjusted at AC voltage of 15 V_{pp}, frequency of 1 MHz and flow rate of 0.3 μL/min. (C) Guiding and capturing performance were applied for 4G2-coated beads. By generating the appropriate nDEP force and drag force, the beads can be captured at the cusp area of the capturing electrode. The guiding function is also

workable for controlling the number of the captured 4G2-coated beads. To achieve both capturing and guiding, the operating parameters of the system were set at AC voltage of 15 V_{pp}, frequency of 1 MHz and flow rate of 0.3 µL/min in DMEM with conductivity of 1 mS/cm.

3.4. DENV detection based on fluorescence intensity on a 3D dielectrophoresis microfluidic chip

The results show that fluorescent signals were clearly observed for DENV concentrations of 10^6, 10^5 and 10^4 PFU/mL at the operating parameters of voltage 15 V_{pp}, frequency 1 MHz, packing and interaction flow rate 0.3 µL/min (Fig. 4B), and no fluorescence signal at the DENV concentrations of 0-10^3 PFU/mL could be observed. Thus, the limit of detection is 10^4 PFU/mL, and due to the rapid reaction time of 3.5 min the on-chip detection time process can be completed within 5 min. However, the total running time on-chip for DENV capture, including chip pretreatment with BSA 1% channel coating (~30 min), 4G2-coated beads packing and DENV capture (~5 min), is approximately 35 min. The volume of DENV sample used on-chip within 5 min with flow rate of 0.3 µL/min for on chip-fluorescence detection was 1.5 µL, and the adjustment volume before detection was roughly 10-15 µL. Therefore, the total volume of the DENV sample utilized to obtain the optimal fluorescence intensity was approximately 15 µL. This is appropriate for platform miniaturization, as recommended to reduce the sample volume to a few microliters (Sin et al., 2014) and even less than 50 µL, which is a volume of required for a clinical sample by some commercial rapid dengue tests (Pal et al., 2014). After quantification according to the protocol shown in Fig. S6, the correlations between the DENV concentrations ranging from 10^4 to 10^6 PFU/mL and fluorescence intensity are shown in Fig. 4A. The negative control with DENV concentration of 0 PFU/mL shows no fluorescent signal while the various

concentration of the DENV shows the higher concentration of the DENV the higher intensity of fluorescence that can be obtained. The fluorescence intensity should increase over time, although aggregation and sedimentation of the DENV mixture caused saturation by time, and thus the fluorescence signal could not increase significantly. It seems that the DENV mixture tends to aggregate over time. The likelihood of antigen-antibody aggregation is caused by the typical nature of an antibody which has bivalent antigen-binding sites (Alberts et al., 2002), thus the aggregation of DENV mixture were considered formed during modification and storage due to the effect of binding sites. For on-chip usage, the DENV mixture was gently shaken by pipetting instead of sonication to disperse an aggregation, however the saturation still occurred in the syringe during injection of the sample at the flow rate of 0.3 µL/min. It was observed that the diameter of DENV mixture aggregates are categorized into 0.8 µm ± 9.7% (<1 µm, N = 10), 1.5 µm ± 29.6% (1-2 µm, N = 10), and 2.4 µm ± 30% (>1 µm, N = 10), determined by the diameter of the single observable fluorescent spot of DENV mixture. Nevertheless, the ability to detect the fluorescence signal shows that the chip platform can capture and detect dengue type 2 virus *in vitro*, which means the platform is applicable to be utilized for rapid dengue virus detection.

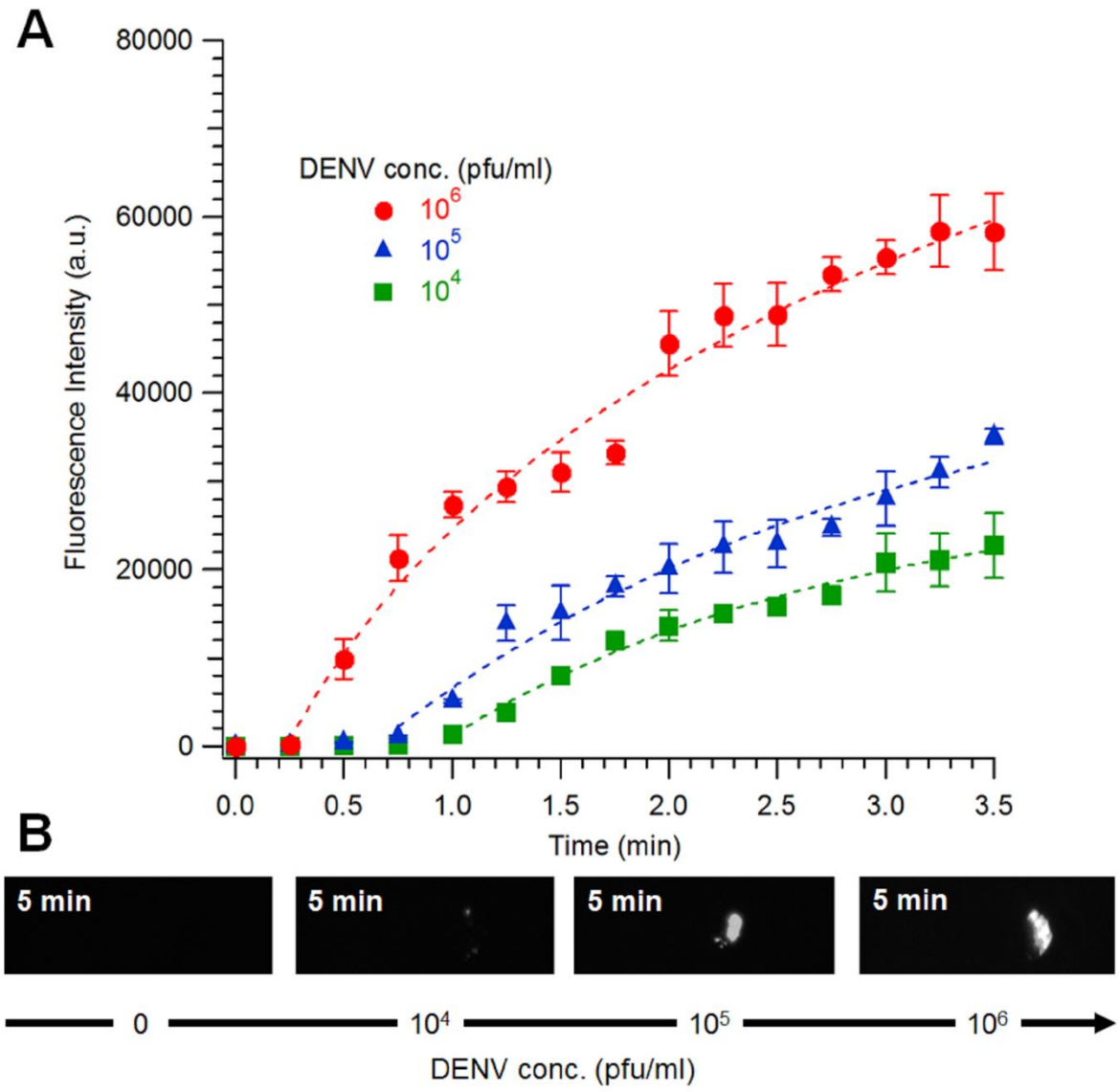

Fig. 4. (A) Correlation between the dengue virus concentrations ranging from 10^4 to 10^6 PFU/mL and fluorescence intensity. (B) The fluorescent signals of dengue virus capturing on the chip. The time obtained fluorescent signals was included the time for capturing of the 4G2-coated beads (~1 min), the time for the injection of the DENV mixture to reach the channel (~0.5 min), and the time for the optimum obtained fluorescent signals (~3.5 min) which is totally 5 min. The sample comprises 4G2-coated beads ($2x10^7$ beads/mL) and DENV conjugated anti-DENV envelope protein antibody labeled donkey anti rabbit IgG-AF 488 with the concentration varied

at 0, 10^4, 10^5 and 10^6 PFU/mL. All of the samples were run in DMEM (σ = 1 mS/cm) at a flow rate of 0.3 mL/min, AC voltage of 15 V_{pp} and frequency of 1 MHz for packing and capturing.

Choosing an ideal dengue diagnostic test depends on the purpose of the examination (Peeling et al., 2010), such as for early clinical diagnosis, surveillance and outbreak investigation, vaccine trial (WHO, 2011) or point-of-care, and each aim will determine the appropriate detection approach. Based on the detection target, conventional dengue infection diagnostic tests are generally divided into direct methods, including viral detection, viral nucleic acid detection and viral antigen detection, and indirect methods, encompassing host antibody response or serology detection (Mardekian et al., 2015; Peeling et al., 2010), such as IgM, IgG and IgA (Blacksell, et al., 2012). Direct methods are recommended for early dengue infection diagnosis within seven days after the onset of symptoms and more confident results can be provided by specificity confirmation. However the accessibility of this approach is still a challenge, as it is expensive and requires well-trained personnel (Guzman et al., 2010), as well as needing from 24 hours to two weeks for viral identification and RNA detection, and 15-20 minutes for NS1 rapid tests (Peeling et al., 2010; Blacksell, et al., 2012). As such, conventional methods of viral detection employing virus isolation by cell culture and viral RNA amplification by PCR/RT-PCR remain the standard approaches (Tang and Ooi, 2012), with sensitivity ranging from 40-100% and a specificity of 100%, while for viral antigen detection these methods have sensitivity varying from 50-93% and specificity from 70-100% (Mardekian, et al., 2015). Undirect approaches, such as serological tests, are widely used because of ease and cost-effectiveness, and thus are commonly applied in surveillance and outbreak examinations, vaccine trials and bedside practice. However, serological tests demonstrate a lack of sensitivity that

varies from 21-99%, with a specificity that varies from 77-98% (Peeling et al., 2010; Mardekian et al., 2015), depending on methods, sample types and picking-time. The assay time needed for dengue rapid diagnostic tests based on antibodies is in the range of 15-30 minutes (Blacksell et al., 2012). On this point, it is obvious that there is no single dengue diagnostic test provides the best performance with 100% sensitivity and specificity (Peeling, et al., 2010).

Based on the capability for detection of DENV particles, this dengue diagnostic platform is suitable to be used during the acute stage, either with symptom or asymptomatic infection. This is because of the high confidence of its results with regard to viral detection, as recommended when choosing an optimal diagnostic approach and time-frame detection (Peeling et al., 2010). Unlike symptomatic dengue virus infection diagnosis during the acute stage, the asymptomatic dengue virus infection is likely to be difficult to diagnose by antigen and serological tests, as the results could be positive or negative due to inactive viremia activities. This issue could be resolved when a diagnostic test can detect dengue virus particles as the source of infection. However, the capability of the system platform should be improved to get a lower limit of detection (LoD), especially for asymptomatic dengue infection diagnosis (Perng, 2015). Prior studies reported that persistent DENV existed by maintaining their low concentration without any active viral replication (Kurane et al., 1990), and without any cytopathic effect of the infected cells (Takasaki et al., 2001), however the number of surviving dengue viral particles in persistently infected humans has not yet been identified. Nevertheless, the platform developed in this work has shown excellent performance, including rapid on-chip detection (5 min) due to a faster reaction time, the low-cost due to less sample being required, and the high robustness during the experiment, with one chip being used approximately 50 times, and this number being even higher if the proper washing, cleaning and drying are conducted after utilization. Therefore,

future work will continue developing this platform to enhance its performance with regard to the limit of detection, specificity, serotype determination, and use with clinical samples.

4. Conclusion

This study presents a proof of concept for a platform carrying out rapid DENV detection *in vitro* which can provide a confident diagnosis of the DENV infection. The beads, antibodies, and fluorescent probes utilized in the designed system were applicable for DENV detection. The platform provides a rapid on-chip detection (5 min) with the small required sample (~15 µL), and the long life-time (>50x reusable), compared to the conventional methods which are commonly time-consuming and need a lot of sample. It is obvious that the developed platform has the potential to be implemented for use with clinical samples to detect DENV infection by improving the limit of detection and specificity. Due to the dangers of DENV epidemics, the benefits offered by this platform with regard to rapid detection and the selection of appropriate treatment, as well as the fact that it is suitable for asymptomatic DENV infection diagnosis, mean that it should be further developed in other studies. Moreover, this technology could be potentially utilized for other immuno-assay-based diagnostic test.

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Appendix A. Supplementary material

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Highlights

- A proof of concept of the platform for detection of dengue virus particle *in vitro* was presented.
- The beads and antibodies utilized in the system were applicable for detection of a dengue virus particle.
- The platform provides rapid detection (5 min) with a low sample volume (15 μl).
- The fluorescence intensity represents the detection of the dengue virus relating concentration.