ON-CHIP NANOMANIPULATION OF SINGLE INFLUENZA VIRUS USING DIELECTROPHORETIC CONCENTRATION AND OPTICAL TWEEZERS

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ABSTRACT

We developed manipulation of single virus using optical tweezers and dielectrophoretic (DEP) force in a microfluidic chip. We used a microfluidic chip made of poly(dimethylsiloxane) (PDMS). The chip has independent virus chamber and cell chamber to make quantitative analysis of the functions of influenza virus before and after infection to a cell. DEP force integrated inside virus chamber concentrates the virus. Concentrated viruses flow to the part for sample selection. The selected virus was transported to the cell chamber using optical tweezers. After virus transport, these chambers were isolated by photocrosslinkable resin. In this paper, we demonstrated DEP concentration of the influenza virus, manipulation of the single virus to transport the cell chamber, contact to a target cell and separation of chambers in the chip.

KEYWORDS: Nanomanipulation, Virus, DEP concentration, Microfluidic chip

INTRODUCTION

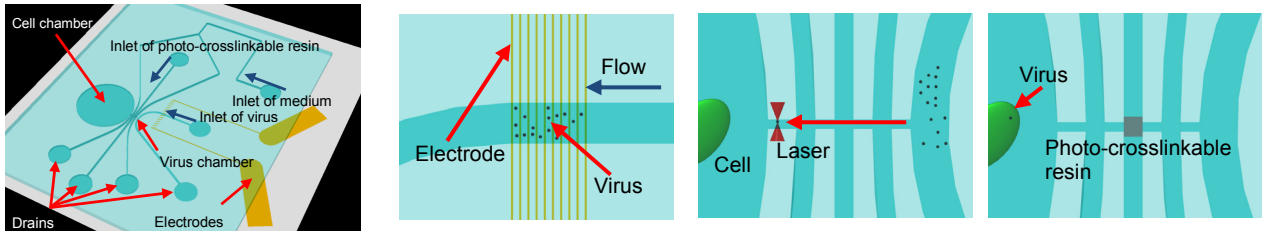
Manipulation of nm-scale biomaterials such as a virus has become one of the most important issues in the nano-biotechnology field in recent years [1]. Conventionally, analysis of the virus functions was performed by using cultured cell and this method was considered as the most precise method [2]. However, this method can only acquire the average information from cells, which are different on physiological state and cell cycle. There are some problems on the conventional understanding using averaged information acquired from a lot of cells because efficiency of virus infection is different in each cell. Manipulation of single virus and infection to the selected target cell are required to achieve the quantitative analysis on the influenza virus. However, manipulation of a single nano-scale biomaterial was difficult. Especially, concentration of sample is one of the most important issues because the influenza virus is the scarce sample.

In this paper, we proposed single virus manipulation using DEP concentration of the virus in a microfluidic chip. This chip has a virus chamber and a cell chamber. Viruses in the virus chamber are trapped and concentrated by DEP force [3]. Concentrated viruses flow to the selection part. The selected virus is transported to the analysis chamber by optical tweezers. We demonstrated DEP concentration of influenza virus, transport of single virus, contact to the target cell in the cell chamber, and isolation of chambers by local photopolymerization in the chip.

THEORY

Figure 1 shows a schematic of a single virus infection to a specific cell in the chip. The chip was composed of the virus chamber, electrodes for sample concentration, the cell chamber and the microchannels for injecting buffer flow and photo-crosslinkable resin as shown in Fig. 1(a). The top of the cell chamber was sealed by self-adhesive material. After the experiment such as virus infection, extraction of the infected cell was performed by open the chamber.

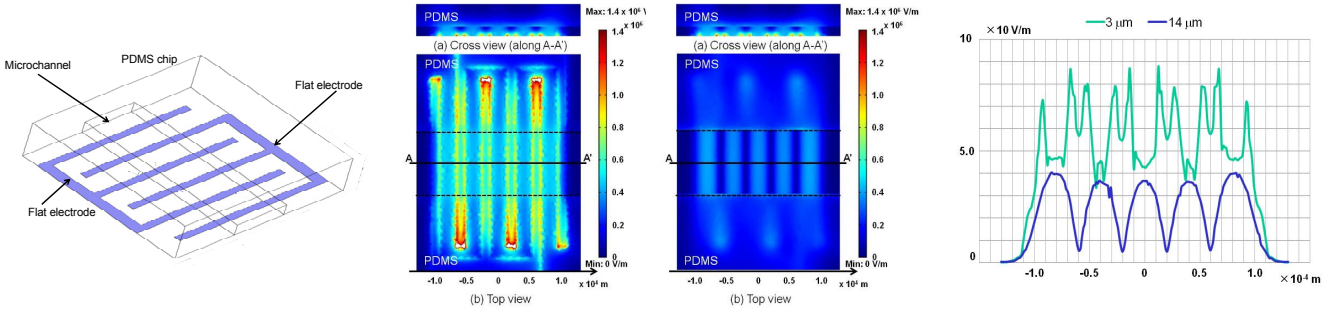
A solution including the viruses was injected into the virus chamber. Process of virus concentration and transport is shown in Figs. 1(b)-(d). Surface of microchannels and chambers were pretreated to avoid adhesion of the virus to the chip. Viruses were stained by fluorescent dye DiI (excitation wavelength: 491 nm, emission wavelength 515 nm) for observation. Each inlet port was connected to the syringe pump. First, the virus solution was loaded to the sample chamber. High- frequency voltage was applied to the electrodes for generating DEP force. The viruses were trapped and concentrated at the part of the electrodes. After concentration of the virus, concentrated viruses flowed to sample selection part. After sample loading to the part for sample selection, single selected virus was trapped by optical tweezers and transported to the cell chamber through three microchannels. Transported virus was released by cut off the laser and used for various experiments such as single virus infection. After transport of the required number of viruses, these chambers could be separated for avoiding entering the other viruses to the cell chamber by stacking these microchannels by local photopolymerization of the photo-crosslinkable resin.



(a) Overall view of microfluidic chip (b) DEP trap of virus (d) Transport of virus (d) Separation of chambers

Figure 1: Schematic of single virus infection to a specific cell

We used negative DEP force for virus concentration because the virus was scaring sample. A schematic diagram of the electrodes was shown in Fig. 2. The width of the electrodes was 10 μm. The gap of the electrodes was 30 μm. The concentration of the virus in a solution was $1x10^6$ viruses/μl. Observation of the virus in the sample chamber was difficult (below 0.1 viruses per field of view). High-frequency voltage between the electrodes generated the DEP force. the particles move toward the direction of low gradient by the negative DEP force.

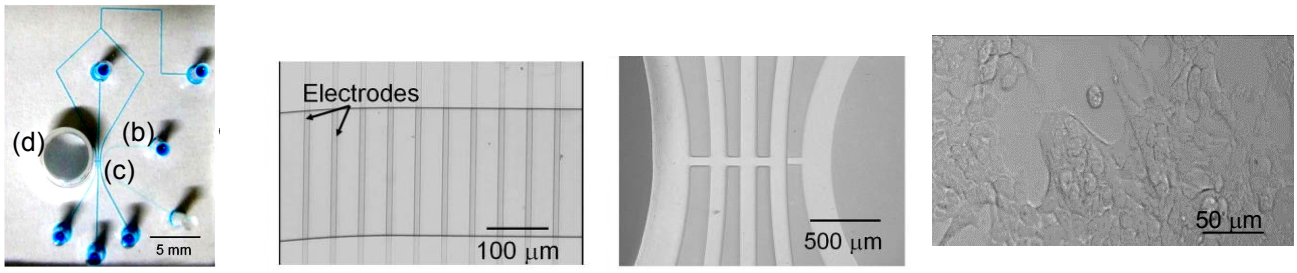


(a) Analytical model (b) 3 μm from electrodes (c) 14 μm from electrodes (d) Distributions of electric field

Figure 2: FEM analysis of electric filed distribution

EXPERIMENTAL

Figure 3 shows photographs of the virus chamber having virus concentration part and sample selection part in the chip. The chip made of PDMS was fabricated by photolithography and replica molding techniques [19]. Mold of the chip was fabricated by multi-exposure to make the chip with the different height areas. First, Cr/Au was sputtered on the Si wafer. Then, positive photoresist OFPR (Tokyo Ohka Kogyo co. Ltd) was spin coated and patterned to make alignment pattern for multi-exposure of negative type resist SU-8 (Kayaku Microchem). After removing the developed OFPR, Cr/Au was etched. SU-8 sheet was coated and patterned. This mold has two different height areas. First area was 15 μm in thickness and was for the virus chamber. Second area was 115 μm in thickness for microchannels and cell chamber. Width of the virus chamber was 200 μm. Width of the other microchannels was 100 μm. Diameter of the cell chamber was 5 mm. Top of the cell chamber was sealed by PDMS membrane sheet to open the virus chamber in case of cell seeding and cell extraction. We could culture the H292 cell in the cell chamber. We used a laser confocal microscope system (A1R, Nikon Corporation) having laser manipulation system (1W, 1064 nm) for DEP virus concentration and single virus infection to the specific cell. Influenza virus was stained by fluorescent dye DiI for virus observation. DiI stains membrane of the virus. First, 1 ml DiI is mixed with 500 ml PBS (-). The mixture is mixed with virus suspension (suspension: virus suspension = 1:1). The virus is incubated in dark for 30 minutes. Finally, virus solution was diluted by adding DI water to adjust the conductivity to 10 mS/m. Virus concentration was 10^6 viruses/μl.



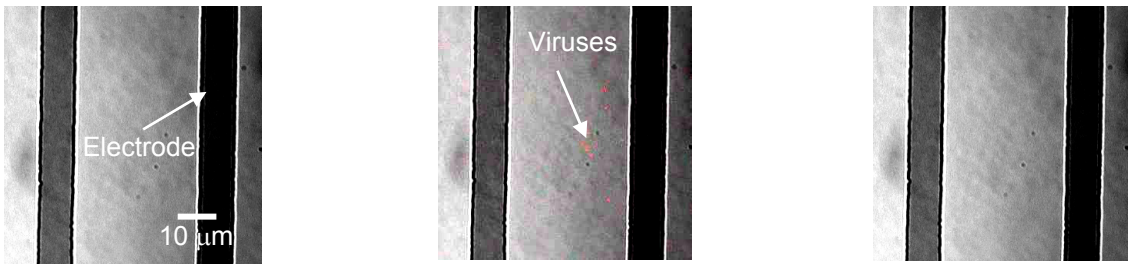
(a) Microfluidic chip (b) Virus concentration part (c) Sample selection part (d) MDCK cell cultured in the chamber

Figure 3: Photo of microfluidic chip

RESULTS AND DISCUSSION

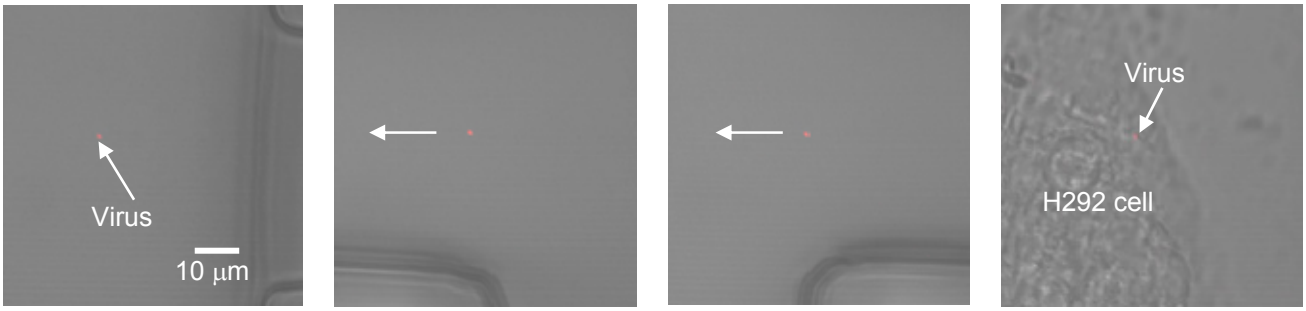
Figure 4 shows the experimental result of the DEP concentration of influenza virus in the chip. The virus was injected to the chip by the syringe pump. Flow rate was adjusted to 0.10 μl/h. We applied square wave to the electrodes. Amplitude was $20V_{p\text{-}p}$. Frequency was 3 MHz. From Fig. 4, concentration of virus was concentrated from 10^6 viruses/μl to10^9 viruses/μl. After DEP concentration of 5 minutes, we could release the virus by cut off the voltage. After release of the viruses, we flew them to the sample selection part. We also confirmed no virus adhered to the glass substrate in the concentration area because DEP force was stronger than Brownian motion of the virus.

Figure 5 shows the experimental result of manipulation of the single virus by direct laser manipulation in the virus chamber using laser micromanipulator (Siguma koki). Laser power was adjusted to 0.5 W. We succeeded in manipulation of the single virus by optical tweezers. Transport speed was approximately 10 μm/s. We transported the virus to the analysis chamber and contact to the selected H292 cell for infection. Finally, the analysis chamber was isolated from virus chamber to avoid incursion of the extra viruses to the cell chamber by local photopolymerization of polyethylene glycol methacrylate (PEG-MA) within 1 second as shown in Fig. 6. From these results, effectiveness of our proposed system for biomedical analysis was confirmed.



(a) Electrodes for virus trap (b) DEP trap of virus (d) Release of trapped viruses

Figure 4: DEP concentration of virus



(a) Trap by optical tweezers (b) Transport of virus (c) Transport of virus (d) Contact to cell

Figure 5: Single virus transport to contact to a specific cell

(a) Before separation (b) After separation

Figure 6: Separation of chambers by in-situ photopolymerization

CONCLUSION

We have developed on-chip single virus manipulation supported by DEP virus concentration for single virus infection to a specific cell. We succeeded in virus concentration from 10^6 viruses/μl to10^9 viruses/μl by negative DEP. Negative DEP force also worked to avoid virus adhesion to the glass. DEP force was useful for on-chip virus manipulation. Throughput of manipulation of biomaterials will be improved by automating this sample concentration. The concentrated virus was manipulated by optical tweezers. We succeeded in making the single virus infection to the specific H292 cell. Transport speed of the virus was about 10 μm/s by direct laser manipulation. This result indicated automation of single virus infection to a specific cell is possible by system integration to the microfluidic chip. Single virus infection is one of the essential techniques for quantitative analysis of the functions of influenza virus before and after infection to a cell. This on-chip single virus manipulation technique will make a great contribution to biomedical applications in the future.

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