FUSION OF VACCINIA VIRUS PARTICLES WITH SINGLE CELLS – A KINETIC STUDY FACILITATED BY MICROFLUIDIC TECHNOLOGY Phillip Kuhn¹, Florian I. Schmidt², Jason Mercer², Petra S. Dittrich¹

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ABSTRACT

We developed a microfluidic device that allows, for the first time, the kinetic investigation of the fusion of single vaccinia virions (VACVs) with single cells initiated by a fast low-pH trigger.

KEYWORDS

Vaccinia virus, fusion, single cells, pH, lag-time

INTRODUCTION

Recent studies have revealed new insights into the entry mechanism of VACVs into host cells. [1] Although it could be proven that VACVs primarily use a process called macropinocytosis for internalization into endocytic vesicles, [2] the exact fusion mechanism of the virus with the endosomal membrane remains unknown. Results from bulk experiments have largely contributed to the understanding of the entry-fusion complex. However, these experiments might obscure virus and cell heterogeneities and often lack high temporal resolution. With the goal of overcoming these shortcomings, we developed a microfluidic device that allows studying the fusion of single VACVs with single cells.

EXPERIMENT

We used a microfluidic device made from poly(dimethylsiloxane) (PDMS) that was bonded to a functionalized cover glass. A 0.4 mg/ml mixture of BSA-FITC/ BSA (1:9) was microcontact printed on the cover glass before the bonding, which serves as a pH sensor. The remaining surface was blocked with BSA. The device incorporated 576 hurdles for single cell trapping (Figure 1). [3]



Figure 1: Schematic of the microfluidic device. a) A PDMS microchip is used to trap single cells labeled with the viruses in geometric hurdles. All fluids are sucked into the chip through a reservoir. b) Cells are trapped next to a spot of BSA-FITC serving as a pH-sensor. c) Side-view of the set-up. Single virus particles are imaged by two-color TIRF microscopy with an evanescent field of 250 nm. The cell is slightly pressed onto the glass by the buffer flow.

Mature virions (MVs) of a normal VACV strain and a non-fusogenic mutant strain (both labeled with a self-quenching concentration of R_{18}) were bound to U937 suspension cells at 4°C. Subsequently, cells were loaded into the chip with a flow rate of 5 µl/min at 37°C. Cells that were not trapped in the hurdles were washed away with PBS.

The fusion process was initiated by changing the pH of the buffer from 7.4 (PBS) to 5 (PBS/MES buffer), and imaged with two-color total internal reflection fluorescence (TIRF) microscopy. Two processes were visualized at the same time: (*i*) the fusion of the membrane by imaging the fluorescence of the membrane dye R18 that is de-quenched upon diffusion into the cell membrane ($\lambda_{exc.} = 561 \text{ nm}$; $\lambda_{em.} > 600 \text{ nm}$) and (*ii*) the decrease of fluorescence intensity of the BSA-FITC spots as a consequence of the pH change ($\lambda_{exc.} = 488 \text{ nm}$; $\lambda_{em.} > 525 \text{ nm}$).

RESULTS

Figure 2 shows fluorescent images of the BSA-FITC spots inside the channel. The fluorescence decreased due to the pH sensitivity of fluorescein. Real time pH sensing is crucial to define the exact time of buffer exchange at the cell immobilization site. The use of microcontact printing enables the dual use of green fluorophores within the experiment. If the whole surface of the glass would be covered with the pH-sensor, the very often-used fluorescent protein GFP could not be used as a content marker for example because the fluorophores would spatially overlap.



Figure 2. Microcontact printed BSA-FITC spots are used as a pH sensor. The graph shows the decrease of fluorescence intensity on the spots due to a change of pH from 7.4 to 5. The data was corrected for background and photobleaching. Fluorescence trace and inset images are acquired by TIRF microscopy. Scale bars: 10 µm

Figure 3 shows a typical fusion experiment. Single virions bound to the cells can easily be identified and tracked over the course of fusion using TIRF microscopy. Cells are kept in position due to the buffer flow. Virus particles do not seem to interact in a significant manner with the BSA coated surface. Interestingly, our measurements show a lag-time of approximately 20 s between the pH drop and the initiation of viral fusion.

The existence of a lag-time has been reported before for influenza virus [4,5] but not yet for VACV. The lag-time has been attributed to conformational changes of the fusion proteins after protonation of side chains.

The control experiment with the mutant VACV strain did not result in a significant increase of R18 fluorescence after the pH change.



Figure 3. VACV fusion with a single U937 cell. a) t = 0: A single cell was imaged by TIRF microscopy. Single VACVs and clusters could be easily observed. b) t = 135 s: After initiation of the fusion, the fluorescence signal increased significantly due to de-quenching of R_{18} . Some dye molecules are already diffusing into the cell membrane. c) t = 540 s: The whole cell membrane is stained with R18. d) Graph of fluorescence intensities of single VACVs and clusters of VACVs over time (mean intensity of indicated circles). The pH recorded with the pH-sensor is also shown. The lag-time between pH change and visible fusion of about 20 seconds is indicated with dashed vertical lines.

CONCLUSIONS

The here presented microfluidic platform, allowing cell immobilization at defined positions and fast buffer exchange, can significantly contribute to the understanding of viral infection mechanisms. Compared to other platforms used for single particle measurements, our method does not require an understanding of the binding mechanism of the virus particles to the cell. This would be needed to use artificial liposomes or lipid bilayers as cell models in the fusion.

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