A MICROFLUIDIC DEVICE FOR REAL-TIME MONITORING OF FLAGELLAR LENGTH IN SINGLE LIVING CELLS OF *CHLAMYDOMONAS*

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ABSTRACT

Chlamydomonas is an extremely powerful model organism for studying cilia/flagella. With traditional methods, *in-situ* monitoring of flagellar assembly/disassembly kinetics in single living cells has been difficult because of time-consuming media exchange, the requirement of whole cell fixation or physical constraints on flagellar dynamics. Here, we develop a PDMS/glass hybrid microfluidic device for real-time tracking of flagellar length in single living cells of *Chlamydomonas*. Complete media exchange is precisely controlled by sequential gas-liquid plugs within seconds. The flagellar kinetics for the whole cell population on the chip was comparable to those from the conventional method, however, individual cells exhibited non-uniform response kinetics.

KEYWORDS

Flagellar length, *Chlamydomonas*, media exchange, real-time monitoring, single-cell analysis

INTRODUCTION

Flagella and cilia are dynamic cell surface organelles whose lengths are carefully regulated, and alterations in length are associated with several human diseases and development disorders, including blindness, polycystic kidney diseases, infertility and situs inversus [1-2]. Green alga *Chlamydomonas* is a widely used model organism to study mechanisms of assembly and disassembly of cilia/flagella. With conventional methods, it usually requires artificial steps as shown in Fig 1a, involving several constraints: flagellar length is measured and presented as average of those from multiple dead cells due to cell fixation; more importantly, transient changes of flagellar length upon extracellular stimuli cannot be captured due to time-consuming process of media switching.

Microfluidics is an emerging powerful methodology for real-time single cell analysis and repeatable media exchange. A few attempts have been made on the analysis of *Chlamydomonas* on chip [3-5]. However, none of these on-chip studies have focused on flagellar length in *Chlamydomonas* at the single cell level. Here, we developed a microfluidic platform for *in-situ* flagellar length studies in single living cells of *Chlamydomonas*.

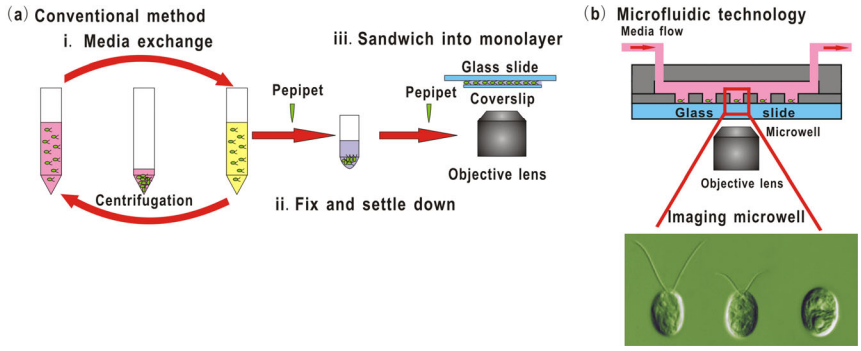

Figure 1. (a) Schematic illustration of conventional method for flagella analysis. (b) Schematic illustration of the microfluidic platform for analysis of Chlamydomonas flagella.

EXPERIMENTAL

The device consists of PDMS/glass hybrid microwells for cell trapping and flagellar imaging (Fig. 1b). Fig 2a shows the procedure of switching media from Solution 1 (pink) to Solution 2 (green) in the microfluidic device. Air plug isolates adjacent liquid plugs, thereby reducing the contamination between neighboring liquid plugs. First, the channel was filled with liquid solution until there were no bubbles trapped inside the microwells. Next, the segmented solution (solution1, 2, 3) and air plugs were pre-loaded into the inlet tubing by suction at the outlet. Then all sequential plugs were introduced into the microfluidic channel by a syringe-pump system. The solution trapped in the microwells can be rapidly replaced by the flowing solution.

To quantify time-lapsed media exchange in the microwells., we introduced 2 µl sequential plugs of fluorescein isothiocyanate (FITC, 1.0µM concentration), air, and pure deionized water (DI water, Millipore) into the channel at 0.5 mL/h. Sequences of optical images of replacing FITC with DI water were obtained.

For pH shock treatment, a series of 2.0 µL gas-liquid plugs containing cells, air, M medium (3 segments, prepared as supplementary), air, acetic acid (pH 4.5, adding trypan blue staining for visualization), air and M medium (3 segments) were injected into the device in sequence. To induce flagellar shortening, cells were incubated in 20 mM NaPPi for 2 h. For flagellar re-growth after induction of flagellar shortening, NaPPi were removed from the cells by fresh M media at different stages of shortening as indicated.

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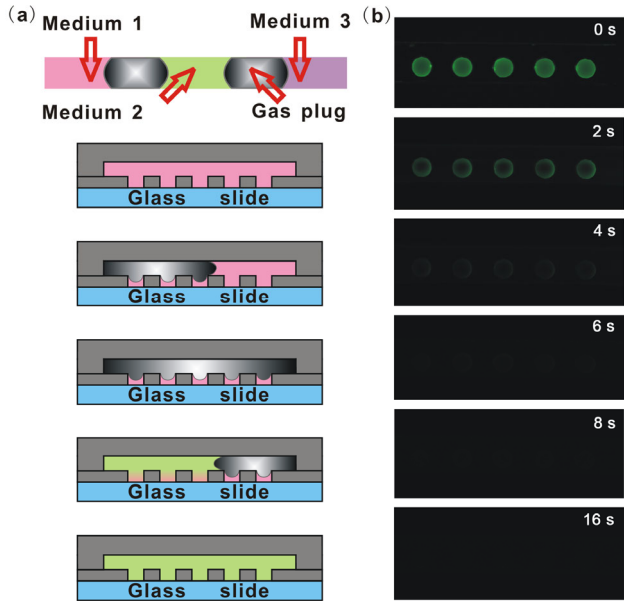

Figure 2. (a) Procedure of switching media from Solution 1 (pink) to Solution 2 (green) by gas-liquid exchange after pre-loading gas-liquid plugs into tubing. Solution 1 (pink) left in the microwells can be completely replaced by Solution 2 (green). (b) A time sequence of optical photographs of the microwells during removal of the fluorescein using a flow rate of 0.5 ml/h at the inlet.

RESULTS AND DISCUSSION

Sequential flow of gas-liquid plugs combined with optimum microwells allow controllable and complete media exchange. The intensity of FITC within the microwells decreased with time, and the fluorescein was completely replaced by DI water within 8 seconds at a flow rate of 0.5 ml/h (Fig. 2b).

To demonstrate the capability of the device for flagellar length studies, we examined the response of flagellar length to different extracellular stimuli. For pH shock treatment, the flagellar lengths increased dramatically after flagellar loss, plateauing eventually. However, individual cells exhibited different flagellar lengths at each certain time point (Fig. 3). Interestingly, we observed that the cells near the center of the microwells were prone to excise their two flagella synchronously, while the cells away from the center shed their flagella asynchronously. This instantaneous dynamics of flagellar length indicate heterogeneity of two flagella as well as the importance of controlling of extracellular environment. To induce flagellar disassembly, cells were treated with 20mM NaPPi for 2 h. As shown in Fig. 4, individual cells showed different flagellar shortening kinetics. Flagellar shortening can be reversed by removal of NaPPi. We found that cells the growth kinetics were different in the cells with shorter flagella that exhibited a faster initial growth rate (Fig. 5). The different response may reflect the ability of cells to dynamically monitor and adjust the length of each flagellum.

The long-term kinetics of flagellar length during flagellar assembly and disassembly on the chip were comparable to those from the conventional method, however, individual cells showed non-uniform response kinetics. These variations may be attributed to cell cycle asynchrony and cellular heterogeneity, highlighting the importance of real-time single cell analysis.

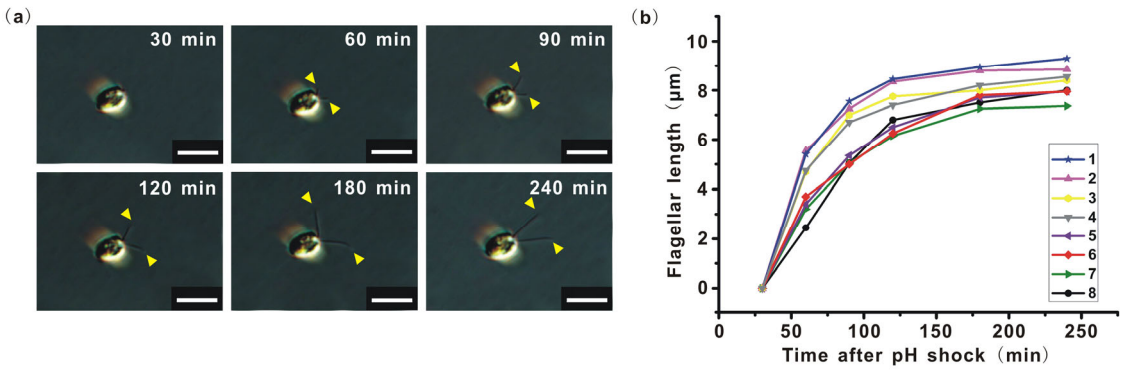

Figure 3. (a) Visual impressions of the time-response upon on-chip flagellar length after pH shock treatment. Yellow arrows point to the flagella tip. Scale bar, 10 μm. (b) Kinetics of flagellar regeneration of individual cells after deflagellation by pH shock. The traces of 8 individual cells are randomly chosen from at least 50 individual cells.

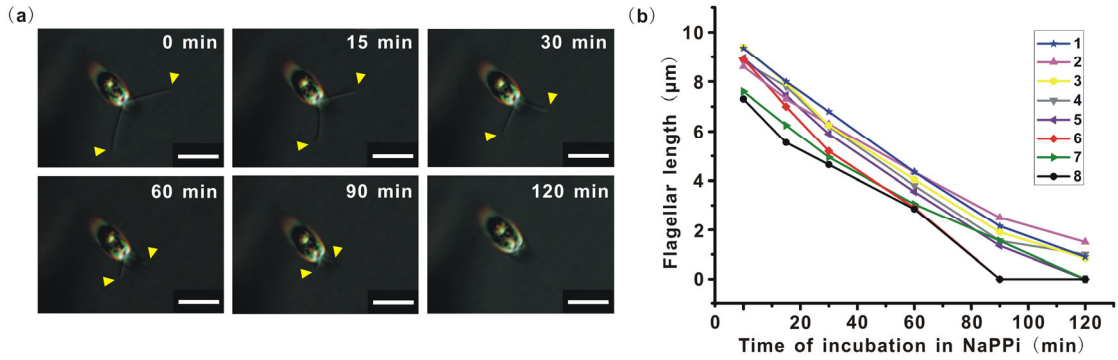

Figure 4. (a) Visual impression of the time-response upon on-chip flagellar length by treatment of 20mM NaPPi for 120 min. Yellow arrows point to the flagella tip. Scale bar, 10 μm. (b) Kinetics of flagellar shortening of individual cells by NaPPi. The traces of 8 individual cells are randomly chosen from at least 50 individual cells.

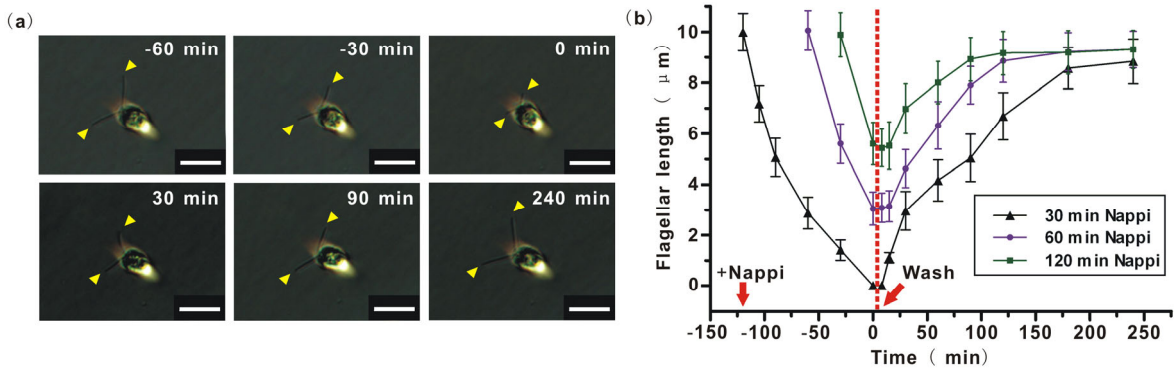

Fig. 5 (a) Time-lapse images of cells undergoing on-chip flagellar shortening and re-growing. NaPPi was added at -60 min and replaced by fresh M medium at 0 min. Yellow arrows point to the flagella tip. Scale bar, 10 μm. (b) Kinetics of flagellar disassembly and re-growth by incubation in 20mM NaPPi for different time scale. Flagellar length from at least 50 individual cells was measured for each time point.

CONCLUSION

Here we introduce microfluidics as a potential platform to control extracellular environment for flagellar length studies. The microfluidic platform herein offers unique advantages over conventional methods. Firstly, it is possible for continuous tracking of flagellar lengths of individual cells throughout their whole lifespan. Secondly, complete media exchange can be precisely controlled, allowing repeatable stimulation and automotive manipulation. Thirdly, the device is low cost with little sample consumption. This microfluidic platform will facilitate flagellar length studies within ciliated cells for better understanding of regulatory mechanisms of flagella; furthermore, it will provide insight into ciliopathy and ciliotherapy.

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