PLANT-ON-A-CHIP MICROFLUIDIC-SYSTEM FOR QUANTITATIVE ANALYSIS OF POLLEN TUBE GUIDANCE BY SIGNALING MOLECULE: TOWARDS CELL-TO-CELL COMMUNICATION STUDY

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ABSTRACT

This paper reports the first observation and quantitative analysis of single cell pollen tube guidance by the signaling molecule realized on the microfluidic device. Design and dimensions of the microfluidic device were optimized for handling individual pollen tube of *Torenia Fournieri*. Concentration of LURE peptide, the signaling molecule for pollen tube guidance, in the reaction chamber was proved to have appropriate gradient for the guidance. Finally, pollen tube guidance by LURE peptide was successfully observed and analyzed under quantitatively controlled conditions.

KEYWORDS

Plant-on-a-chip, Pollen tube guidance, Cell-cell communication, Microfluidics, PDMS

INTRODUCTION

Various elements involved in plant reproduction have been identified so far, and led us to deep understandings of their mechanisms. Recently, various secreted peptides (polypeptides) have been reported to be involved in cell–cell communication of pollen–pistil interactions [1]. For example, defensin-like polypeptide LUREs were proved to be the pollen tube attractants secreted from synergid cells [2]. However, quantitative studies in communication mechanisms between pollen tube and pistil has not been enough studied. This is mainly due to the difficulties in quantitative analysis of pollen tube elongation; pollen tubes disorderly elongate on conventional agarose gel medium [3]. Microfluidic device is one of the attractive candidates for the future platform of their physiological study since it allows not only precise handling and quantitative analysis in pollen tube itself but also in signaling molecules. Pollen tube guidance in plant reproduction was studied by microsystem-based assay, however, handling and observation of individual pollen tubes have not yet be achieved [4]. Thus, we developed our own microfluidic-system to handle individual pollen tubes to realize quantitative analysis of single pollen tube behavior.

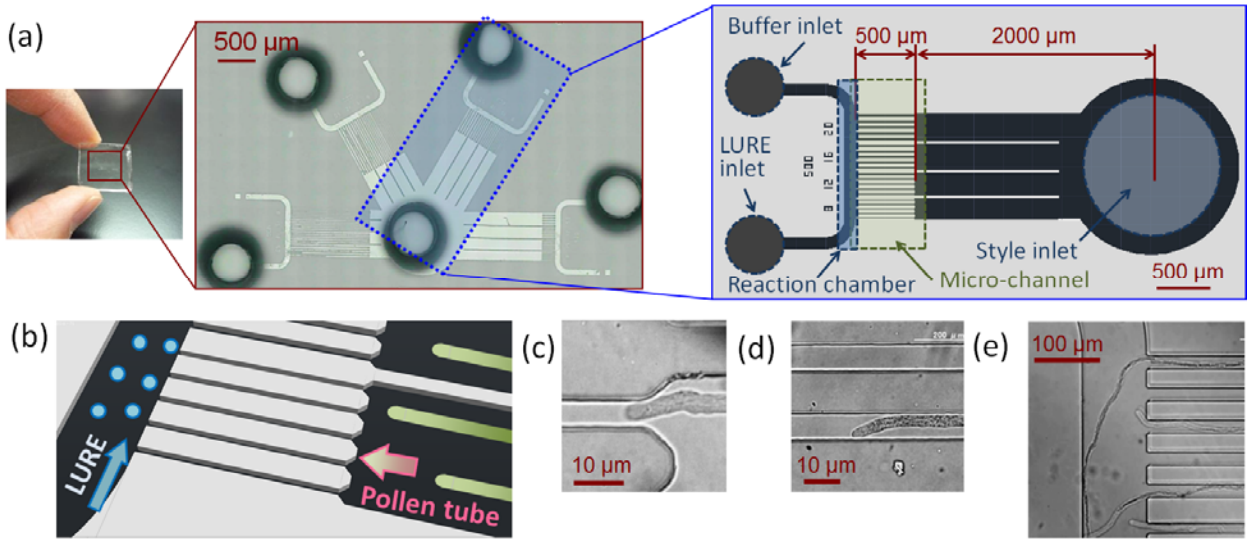

Figure 1. Schematics of the microfluidic device. (a) Microscopic view and schematics of the microfluidic device. Microfluidic device consists of microchannels and reaction chamber. Microchannels are 10 µm in height, and 8, 12, 16 and 20 µm in width. (b) Schematics of pollen tubes entering the microchannels. (c) A pollen tube entering an 8 µm width microchannel. (d) A pollen tube elongation in a 12 µm width microchannel. (e) Pollen tubes entering the reaction chamber.

EXPERIMENTAL

The poly-dimethylsiloxane (PDMS) microfluidic device consists of microchannels, which isolate and parallelize individual pollen tubes, and a reaction chamber in which pollen tubes interfere with signaling molecules (Figure 1 (a) (b)). Microchannels are 10 µm in height, 500 µm in length, and 8, 12, 16 and 20 µm in width. Style inlet, LURE inlet and buffer inlet of 1.5 mm in diameter were developed by punching out the PDMS sheet manually. The distance between the center of style inlet and microchannels is 2 mm. The reaction chamber is 100 µm in width and 900 µm in length.

To confirm that the signaling molecules, supplied from the LURE inlet, can remain appropriate concentration gradient in the reaction chamber, time dependence concentration gradients in the reaction chamber was theoretically estimated by Finite Element Method using a commercial soft-ware (Coventor). Diffusion coefficient was set to 100 $\mu m^2/s$, which is the coefficient for 10 kDa molecule, approximately the same size than LURE peptide. 1 nM molecule was continuously supplied from the LURE inlet in diffusion dominant condition. Diffusion is the dominant transport mechanism and convection can be considered minimal. Therefore, convection was not considered in the simulation. Supplied molecule was assumed to keep steady-state concentration in the LURE inlet. Diffusion of molecule was assumed to take place in an infinite medium from style inlet and buffer inlet.

Experimental protocols were as follows. First, a microfabricated PDMS sheet was placed on the middle of a glass-bottom dish. Second, after degassing the device in a vacuum chamber at 10 kPa for at least 40 minutes, 20 µL of liquid medium was injected from the style inlet to fill all the channels by self-stand pumping mechanism of PDMS microchannel device [5]. Third, a pollinated style (pistil) of *Torenia Fournieri*, cut into 2 cm length, was set in the style inlet of the device. Fourth, six hours after a pollinated style of *Torenia Fournieri* was set into the style inlet, 1 nM LURE peptide contained buffer was supplied to the LURE inlet. Finally, four hours after LURE peptide was supplied, when pollen tubes pass through the microchannels and about to reach the reaction chamber, behavior of pollen tubes guided by LURE peptides was observed by the bright field microscopy.

RESULTS AND DISCUSSION

Pollen tubes were individually isolated, passed through the microchannel and reached the reaction chamber (Figure 1 (c) (d) (e)). Multiple pollen tubes could enter a microchannel of more than 12 µm in width. On the other hand, even a single pollen tube could not enter a microchannel of less than 6 µm width. Channel size dependency of pollen tube behavior showed that the appropriate channel width for single tube isolation is 6~12 µm with the height of 10 µm (Table 1).

Table 1. Channel width dependent number of pollen tube entering a microchannel.

Channel width	Number of pollen tube
<6 µm	No entry
6-12 µm	1
>12 µm	>2

Simulation results showed that the concentration of LURE peptide in the reaction chamber forms linear gradient and keeps enough gradient at least for six hours (Figure 2). When 1 nM LURE peptide was added from the LURE inlet, the concentration of LURE forms linear gradient from 0.66 nM to 0.23 nM in the reaction chamber. Since preferable concentration of LURE peptides for pollen tube guidance is known to be between 4 nM and 40 pM [6], this condition also meets the optimal range in concentration.

The pollen tubes were successfully guided to the direction of higher LURE concentration in the reaction chamber. Figure 3 shows snapshots of an elongating pollen tube, which passed through 8 µm width microchannel, in the reaction chamber towards the direction of higher LURE concentration. For statistical analysis, three domains in the reaction chamber were defined as follows (Figure 4). A right domain out of the 15 degrees line from exit of microchannel was defined as “Right”, a left domain out of the 15 degrees line as “Left”, and a domain in between the two as “Middle”. LURE peptides were supplied from “Right” direction in the reaction chamber. The statistical analysis showed significant

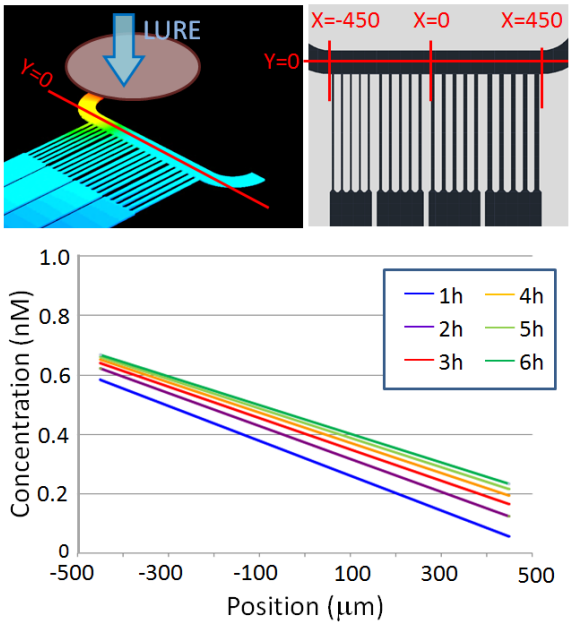

Figure 2. Distribution of LURE peptide in the reaction chamber one to six hours after the injection of LURE contained buffer.

difference between the “Right” domain and the “Left” domain for the number of pollen tubes went towards (Figure 4). On the other hand, significant difference could not be found when the experiment was repeated without supplying LURE peptides; pollen tubes elongated to three domains in random order. Thus, this system realized real-time observation of pollen tube guidance in single cell level in quantitatively controlled conditions.

CONCLUSIONS AND OUTLOOK

Design and dimensions of the microfluidic device were optimized for handling individual pollen tube of *Torenia Fournieri*. The appropriate channel width for single tube isolation was 8~12 μm with the height of 10 μm. Theoretical estimation showed that the concentration of LURE in the reaction chamber forms linear gradient enough for pollen tube guidance at least for six hours. The pollen tubes were successfully guided to the direction of higher LURE concentration in the reaction chamber which is the first observation of plant cell response to signaling molecule with quantitatively controlled conditions.

This on-chip observation of interaction between pollen tube, which is a single cell, and signaling molecules might be the pioneering work for future plant-on-a-chip study. Further quantitative study might elucidate important knowledge for understanding cell-to-cell communication, one of the most important topics in the next generation of biology.

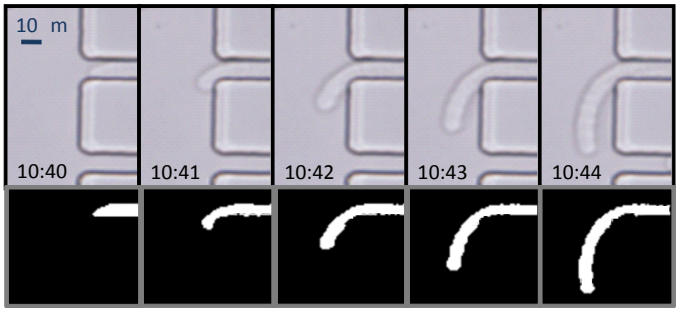

Figure 3. Snapshots of an elongating pollen tube in the reaction chamber towards the direction of higher LURE concentration (downward). Black and white cutouts show changing form of the pollen tube.

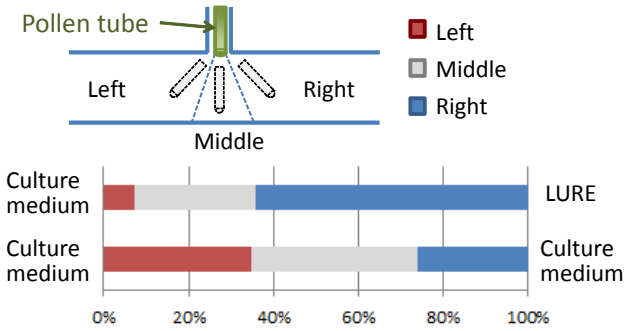

Figure 4. Statistics of the pollen tube guidance. Elongation direction showed prominent tendency towards high LURE concentration.

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