Applications of Microfluidics for Studying Growth Mechanisms of Tip Growing Pollen Tubes

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Abstract— **Pollen tube, the fastest tip growing plant cell, plays essential role in life cycle of flowering plants. It is extremely sensitive to external cues and this makes it as a suitable cellular model for characterizing the cell response to the influence of various signals involved in cellular growth metabolism. For in-vitro study of pollen tube growth, it is essential to provide an environment the mimics the internal microenvironment of pollen tube in flower. In this context, microfluidic platforms take advantage of miniaturization for handling small volume of liquids, providing a closed environment for *in-vitro* single cell analysis, and characterization of cell response to external cues. These platforms have shown their ability for high-throughput cellular analysis with increased accuracy of experiments, and reduced cost and experimental times. Here, we review the recent applications of microfluidic devices for investigating several aspects of biology of pollen tube elongation.**

I. Introduction to Pollen Tube

Pollen tube is the fastest tip growing plant cell that plays essential role in life cycle of flowering plants. It germinates from pollen grain on stigma, senses multiple extracellular mechanical and chemical signals from its floral environment and elongates in polarized shape toward the ovule in the interior of the flower. It has the function to deliver the migrating sperm cells to the eggs for fertilization purpose. Pollen tube grows through distinctive cellular matrix as the tip growing cells follow properly the guidance cues.

Pollen tube is extremely sensitive to the external cues and this makes it as a suitable cellular model for characterizing cell response to the influence of various signals involved in cellular metabolism. For *in-vitro* study of pollen tube growth mechanism, it is essential to provide an environment the mimics the internal microenvironment of pollen tube in flower. Conventional plant cell analysis methods rely on culturing the seeds, growing the plant cells in soil pots or agarose plates, followed by screening the plant phenotypes in traditional greenhouses and growth chambers. These methods are usually costly, need a large number of experiments, and suffer from information loss during the monitoring of the plant phenotypic changes due to the low temporal resolution.

Micro-electro-mechanical systems (MEMS) technology has been widely used for biological studies in the last decade, led to development of BioMEMS, with different applications in areas of drug development, biosensing, synthesizing *in-vitro* organs, and particularly, for cellular studies. Microfluidic platforms, which provide closed microenvironments, take advantage of miniaturization for handling small volume of liquids for *in-vitro* single cell analysis and characterizing the cell response to different external cues [1]. In addition, the ability of high-throughput cellular analysis increases the accuracy of experiments, and reduces the cost and experimental times. Despite extensive research on animal cells within microfluidic environments their application for plant cell studies has not been accomplished yet [2]. In this context, microfluidic platforms has recently gained attention for studying pollen tube as a model for tip growing plant cells. Here, we review the recent application of microfluidic devices for investigating several aspects of biology of pollen tube elongation.

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II. TipChip: The Microfluidic Platform for Studying Biology of Pollen Tube

TipChip, as an integrated microfluidic platform, was developed to characterize the growth of tip growing plant cells and particularly, the pollen tube [3]. From design perspective, the TipChip is made of two PDMS layers permanently bonded under plasma activation. The bottom layer consists of a series of growth microchannels connected to fluidic inlet and outlets to allow elongation of individual pollen tubes along the microchannels. Two main outlets are responsible for conducting pollen tubes to be trapped at the entrance of microchannels. The rest of pollen grains are directed toward the outlet drain (Fig. 1a) [3]. After entrapment of pollen grain, the pollen tubes start germination, followed by elongation in horizontal plane along microchannels (Fig. 1b). The TipChip was tested to study growth behavior of *Camellia* pollen tube. The results showed that both germination rate and growth rate of pollen tubes elongating within microfluidics were comparable with the results achieved from conventional *in-vitro* assays. This confirms that the TipChip can provide a suitable environment for studying serially arranged tip growing pollen tubes with the purpose of targeting several unknown biological questions.

The dimensions of the TipChip were optimized to ensure that only one or two pollen grains are trapped at each microchannel and to provide identical growth condition along all microchannels as prerequisite for further chemical simulation or toxicity testing [4]. It was demonstrated that

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the pollen tubes are very sensitive to the fluid velocity within the microchannel and thus excess flow velocity may cease the tube elongation or cause cell bursting [3]. Due to the structure of serially arranged microchannels, different biosensors such as microgaps or microcantilevers were later integrated along the microchannels to detect the response of pollen tube to external chemical, mechanical or electrical signals [5].

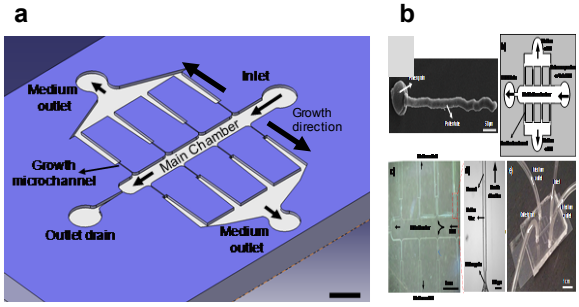

Figure 1. TipChip platform, a) A schematic of TipChip design. Pollen suspension injected into the chip are transported through the main chamber toward the growth microchannels. The leftover pollen grains are evacuated into the outlet drain, Scale bar: 1 mm, b) A sample pollen tube elongating straightly along a gowth microchannel

III. Characterizing Oscillatory Dynamic of Pollen Tube Elongation

Pollen tubes do not elongate in a steady fashion, but display oscillatory dynamic growth [6]. This oscillatory behavior has an impact on regulatory mechanism of growth and is correlated with oscillatory behavior of several other parameters involved in growth regulation. The growth oscillation is subjected to modulation in response to external triggers. The temporal and spatial resolution of previous open assay experiments prevented accurate characterization of the oscillatory growth pattern. These experiments had mainly focused on measuring variation of average growth rate, oscillatory period, and oscillation amplitude under chemical stimulation [7]. Several studies investigated the oscillation frequency as one characteristics of dynamic growth in pollen tubes [8]. While both primary and secondary oscillatory frequencies of ion transport have been detected around pollen tube's tip, the open assays have mainly detected the primary peak oscillation frequency of growth and its variation under chemical treatment. To overcome the limitation of detecting higher mode oscillation frequencies, we used the modified TipChip design for high resolution imaging of the *Camellia* pollen tube elongating within liquid medium without its displacement during imaging process [6]. Using this platform, the existence of both primary and secondary peak frequencies and their variations under the effect of different sucrose concentrations were detected (Fig 2). It was also illustrated that even in apparent absence of low frequency oscillations in young pollen tubes, the secondary peak frequencies always exist, indicating that pollen tubes experience a dynamic growth. Both primary and higher mode oscillatory frequencies were shifted under the sucrose treatment, indicating how pollen tube regulates its growth behavior at different modes of oscillation. The relation between these frequencies and other physiological processes, such as the oscillation of extracellular and intracellular ion gradients, and ion fluxes can be further studied within microfluidics for better understanding of pollen tube biology [8].

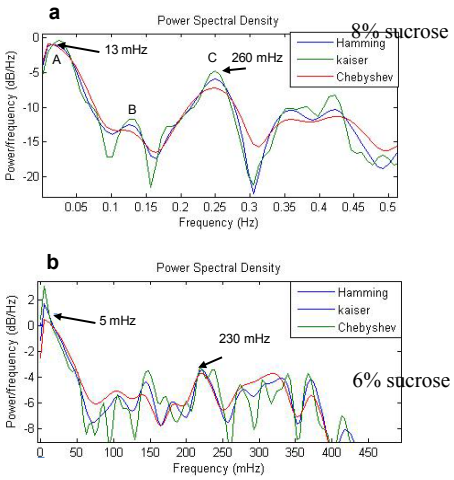

Figure 2. Detection of shift in both primary and secondary oscillation frequencies under treatment with different sucrose concentrations. a) Primary and secondary frequencies of pollen tube in normal 8% sucrose concetnration, b) the shift in both primary and sconcdary frequencies under treatment with 6% sucrose concentration [6]. PSD analysis was subjected to three different windows of Hamming, Kaiser and Chebyshev.

IV. Quantifying Mechanical Properties of Pollen Tube's Cell Wall

To model pollen tube growth, it is essential to obtain quantitative values for mechanical properties of cell wall. Pollen tube's cell wall is surrounded by rigid polysaccharides. Due to microscopic size of individual pollen tubes, the direct measurement of mechanical properties of cell wall has been challenging. Indirect methods of tensile testing on plant tissues measured by instron instrument estimated mechanical properties of plant cell walls [9]. The alternative technique of pressure probe was used to roughly estimate mechanical properties of cell walls [10], but it is not accurate for characterization of subcellular resolution of cell wall. Direct techniques of micro-indentation and atomic force microscopy have been exploited [11], however, due to local manipulation of cell wall, the estimated value for Young's modulus may not be reasonably correspond to highly anisotropic cell wall made of complex composition of multiple polysaccharides [12]. Mechanically speaking, to measure tensile strength of plant cell wall, it should be subjected to tensile or bending loads that is technically challenging due to small size of most plant cells [13].

Microfluidics has played role to implement bending test on pollen tube to quantify the Young's modulus of cell wall using a device called, Bending-Lab-On-Chip (BLOC) [14]. The design of BLOC is similar to the TipChip. Pollen suspension is introduced into the chip while pollen tube elongating along the growth microchannel, is exposed to a bending fluid loading (Fig 3a). Numerical modeling was employed to model the pollen tube bending under fluidic loading and to extract elastic modulus of cell wall (Fig 3b). The value of the cell wall's Young's modulus was in

agreement with the results of pressure probes and the values estimated from the testing on the reconstituted cellulose-callose material as the main polysaccharides of pollen tube cell wall [14]. BLOC technique is a direct method to measure elastic modulus of cell wall and thus, has the advantage of presenting a global value for mechanical properties of cell wall independent from local anisotropies of cell wall.

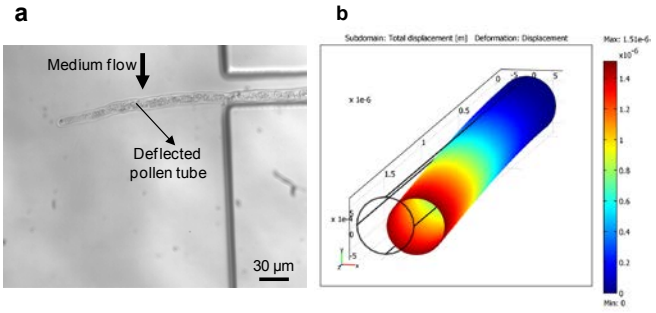

Figure 3. Estimation of Young's modulus of pollen tube cell wall by Bending-Lab-on-Chip (BLOC), a) Pollen tube bended within the microchannel under fluid flow loading, b) Nmerical analysis of pollen tube bending to estimate elastic modulus of cell wall [14].

V. Local Chemical Stimulation of Pollen Tube's Tip

Pollen tube is a very sensitive plant cell model to chemical signals, as it responds to chemical triggers by changing elongation rate or growth direction [15]. Pollen tube has symmetric growth pattern under analogous growth conditions around its apical domain, but exposure of pollen tubes to local asymmetric triggers can disrupt straight elongation. Local cues can be generated by ovule in form of electrical signals or chemical gradients attracting or repelling the pollen tube [16]. There is still a high biological interest to realize what agents are involved in reorienting growth direction, either attractive or repulsive, and how cellular mechanisms of growth are regulated by external chemical signal for this redirection. Developing new techniques for precise manipulation of elongating pollen tube at subcellular resolution is very desired to locally apply different concentrations of growth inhibitors or promoter agents to the growing site of pollen tube. Microfluidics has been used for *in-vitro* study of pollen tube redirection toward ovule [16]. In this device, the pollinated pistil is placed in microgrooves, while pollen tube elongates from pistils and grow through microchannels and toward two side chambers. One of the side chambers is occupied by ovules, while opposite chamber is left empty. This configuration generates asymmetric concentration around pollen tube's tip and causes a turn on pollen tube growth toward ovules, suggesting that pollen tube senses a concentration gradient from the virgin ovules site [16].

Although embedded ovules placed within microsystem proved the presence of a chemical signal from the ovules to the pollen tubes, the type of agent and its concentration gradient is difficult to detect. To overcome this challenge, a microfluidic device was developed to provide precise local concentration of agents [17]. Local chemical administration to a pollen tube at subcellular resolution has successfully been implemented using laminar flows in the TipChip [3]. In Addition to the laminar flow-based gradients, the diffusive gradient was recently exploited to expose pollen tube of Torenia Fournieri to the concentration gradient of signaling molecules, LURE peptide, in order to present quantitative analysis on pollen tube guidance [18]. The results show that the pollen tube was guided toward higher LURE concentration in the reaction chamber.

VI. Measuring Growth Force and Investigating Mechanism of Invasive Growth for Pollen Tube

Microfluidics has also been used to investigate the interaction of pollen tube and surrounding tissues to better understand the invasive lifestyle of plant cells [19]. Pollen tubes elongate through extracellular tissue or matrix to perform their biological task, however, the cellular mechanisms involved in invasion to tissues has not been fully explored. The pollen tubes are hypothesized to use two strategies in combination to invade their extracellular matrix: 1) generating enzymes locally to soften tissue or matrix at their growing site, 2) exerting invasive force to deform the tissue [20]. The driving force required for both elongation and invasion is generated from internal turgor pressure. Only a portion of turgor pressure can be exerted to the surrounding tissue (invasive force) and significant part of this driving force is used to overcome cell wall stiffness and to yield the cell wall for continuous elongation [21]. Agarose-stiffened growth matrix has been used to qualitatively determine capability of pollen tube for invasion. However, to obtain quantitative values for invasive force requires integrating sensors in subcellular size which is difficult to develop using conventional testing assays. Conventional strain gauge was placed perpendicular to growth direction of fungal hyphae in order to quantify the growth force. However, due to change of cellular shape under the contact with the gauge sensor and the redirection of growth, measured values were not conclusive.

We have recently developed a microfluidic device to investigate penetrative force of pollen tube [5]. In this device, the TipChip design was adapted by incorporating mechanical constricts along the growth microchannels. Individual pollen tubes elongating through the microchannels interact with microgaps made of polydimethylsiloxane (PDMS), resembling the apoplast of the transmitting tissue. The results of interaction show that pollen tubes are able to exert their penetrative force (wedge force) into the gaps and deform these micro-constricts [5]. It was also observed that some of pollen tubes burst during interaction with PDMS obstacles. This raised the argument that pollen tubes regulate their cell wall mechanical properties (softening cell wall at the apex) in order to generate sufficient invasive force to pass through the gaps. We also used a simpler technique for accurate quantification of growth force (Fig. 4). The pollen tube interacted with a flexible PDMS microcantilever and applied its growth force to the beam. The beam deformation was measured using

optical microscopy and image analysis and was used to quantify the growth force. The amount of growth force estimated from analysis on vertical PDMS microcantilever was very similar to the value quantified from the interaction with microgaps [5].

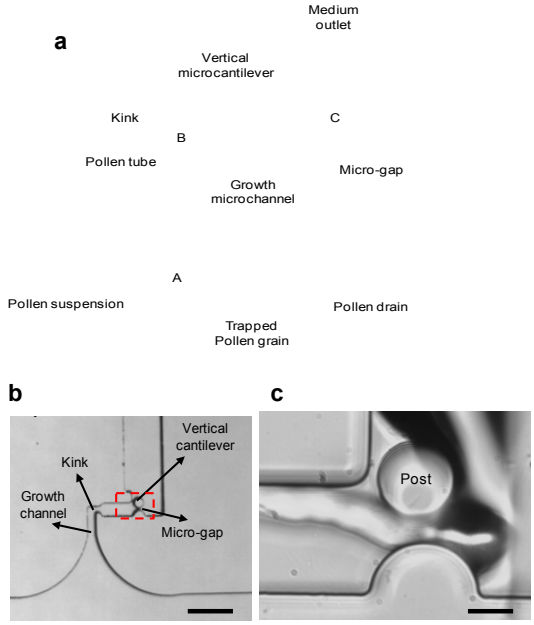

Figure 4. The integrated vertical PDMS microcantilever within microfluidic chip to measure growth force of pollen tube. Growth force was quantified equal to 1.6 μN.

VII. Current Challenges and Future Direction

The aforementioned microfluidic platforms were developed to approach different aspects of pollen tube biology. These platforms can be further adapted to study other biological parameters involved in pollen tube elongation. In context of pollen tube-ovule communication, other molecular cues, nominated to be effective on pollen tube reorientation, can be locally applied to the pollen tube tip. To know how water uptake and ion fluxes through not the apical region, but the distal region of pollen tubes regulates the growth mechanism, novel microstructures embedded within microfluidics will be required. The Bending-Chip is expected to be further modified to quantify the anisotropy of cell wall mechanical properties and to investigate the effect of drug or toxic agents on cell wall softening. In this case, pollen tube can be subjected to bending fluid loading in subsequent testing chambers, where each chamber stimulate a portion of pollen tube exposed differently to a drug or toxic agent. Also, several other biological parameters involved in the invasive life style of pollen tube such as the friction force between growing tip and extracellular matrix, and digestion of extracellular matrix by enzymatic activities can be further studied using novel microfluidic designs.

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