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# Characterization of size-dependent mechanical properties of tip-growing cells using a lab-on-chip device†

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## Supplementary text

### TEM Protocol

Pistils of mature dehiscent flowers are pollinated. 28 hours after pollination, the pistils are cut with a blade to about 1.3 mm into the style of the flower, followed by perforation. It is then fixed overnight at 4 °C in 1.25% glutaraldehyde with a 0.1 M cacodylate buffer. The fixed samples are washed 5 times in Milli-Q water for 10 minutes each. The lower ends of the pistils are then cut into 3 mm pieces and fixed in 1% osmium tetroxide solution for 3 hours. The samples are again washed 5 times in Milli-Q water for 5 minutes each followed by 10 minutes of serial dehydration in acetone solutions with concentrations of 30%, 50%, 70%, 90%, 100% and 100% extra dry acetone. Dehydrated samples are then placed in 50% Epon resin/50% acetone for 3 hours after which the transmitting tract epidermis is peeled off and transferred into 100% Epon resin. They are then cured at 60 °C for 48 hours. Finally, the cured blocks are trimmed and ultra-thin sections are collected on grids, contrast-stained with uranyl acetate and lead citrate, and visualized by the FEI CM100 Transmission Electron Microscope (Philips).

### Automatic Pneumatic Control System

The pneumatic control of the microvalves is realized by controlling compact, direct acting, 3 port solenoid valves (USG2-M5-DC12V, CKD Corporation, Japan). These valves control the opening and closing of the microvalves electronically. The compressed air is supplied by a nitrogen tank and the pressure is regulated by a mounted pressure gauge with a resolution of 5 mBar (500 Pa). The solenoid valves are controlled by a Universal Serial Bus (USB) 16 relay board (Denkovi Assembly Electronics LTD) and a LabView program developed for communication between the relay board and the computer.

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† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

### Turgor Pressure based on Permeability

The turgor pressure in plant cells is created by the movement of water across the semi-permeable plasma membrane due to a concentration gradient between the interior of the cell and the external medium. This process is governed by the following equation

$$\frac{dV}{dt} = -L_p A(\delta T - \delta\pi),$$

where $V$ is the internal volume, $t$ is the time, $L_p$ is the hydraulic permeability, $A$ is the membrane area, $\delta T$ is the hydrostatic pressure difference and $\delta\pi$ is the osmotic pressure difference [1,2]. Expanding the $\delta T$ and $\delta\pi$ terms gives

$$\frac{dV}{dt} = -L_p A[T - RT_p(C^i - C^o) - RT_p({C_s}^i - {C_s}^o)],$$

where $R$ is the gas constant, $T_p$ is the temperature, $C$ and $C_s$ represent the concentration of non-permeable (water) and permeable solutes, respectively, and the subscripts $i$ and $o$ denote the interior of the cell and outer culture medium respectively. Since we are interested in the equilibrium turgor pressure, the temporal derivative of cell volume ($dV/dt$) is set to 0. This leads to

$$\frac{T}{RT_p} + C^o + {C_s}^o = C^i + {C_s}^i.$$

Expanding the concentration terms gives

$$\frac{T}{RT_p} + \frac{4(n_o + {n_o}^s)}{V_T - \pi {D_0}^2 L} = \frac{4(n_i + {n_i}^s)}{\pi {D_0}^2 L},$$

where $n$ is the number of moles, $V_T$ is the volume of the container, and $D_0$ and $L$ are the diameter and length of the pollen tube respectively. Contracting these terms leads to

$$\frac{T}{RT_p} = \frac{4[(n_i + {n_i}^s)(V_T - \pi {D_0}^2 L) + (n_o + {n_o}^s)(\pi {D_0}^2 L)]}{\pi {D_0}^2 L(V_T - \pi {D_0}^2 L)}.$$

Dividing by $V_T$ and using the total number of moles in the system $N_T = n_o + n_i$

$$\frac{T}{RT_p} = \frac{4[(n_i + {n_i}^s) - (\frac{N_T}{V_T} + \frac{{N_T}^s}{V_T})\pi {D_0}^2 L]}{\pi {D_0}^2 L(1 - \frac{\pi {D_0}^2 L}{V_T})}.$$

Assuming $V_T \gg \pi {D_0}^2 L$ leaves

$$\frac{T}{RT_p} = \frac{4[(n_i + {n_i}^s)}{\pi {D_0}^2 L}.$$

From the above equation, it is clear that $T$ must be inversely proportional to ${D_0}^2$. Furthermore, the relation between $T$ and $D_0$ must depend on the osmolarity of the growth medium.

**Closed-Form Solution for a Pressurized Cylinder under Concentrated Opposite Loads**

The elastic part of the closed-form solution for the deformation of a cylinder with an internal pressure subjected to concentrated lateral loads (like rods) is given by[3]

$$Q = \frac{4.48Eh^3}{(1-\nu^2){D_0}^2}(1 - \frac{T}{T_e})x,$$

where $Q$ is the lateral load, $x$ is the penetration displacement, and the elastic buckling pressure $T_e = 2Eh^3/(1-\nu^2){D_0}^3$. The original model assumed perfectly elastic behavior prior to the formation of plastic hinges, with plastic behavior dominating after the activation of collapse. Since the pollen tube is considered to be perfectly elastic in our study, we disregard the plastic part of the solution. This rod-like deformation was chosen as it more closely resembles the deformation of the PDMS membrane compared to the plate-like indentation. This is because the PDMS layer is glued along its boundaries to the top of the compression microchannel (bottom layer of chip), resulting in a clamp-like constraint. The majority of the PDMS deformation is therefore in the center region of the channel, thereby compressing the tube mostly around its central axis, like a rod.

Integrating $Q$ over the length of the tube $L$ gives the applied pressure $P$

$$P = \frac{4.48Eh^3}{(1-\nu^2){D_0}^2}(1 - \frac{T}{T_e})xL.$$

Converting the penetration displacement $x$ to the lateral diameter $D_l$ gives

$$P = \frac{4.48Eh^3}{(1-\nu^2){D_0}^2}(1 - \frac{T}{T_e})\sqrt{1-(D_l/D_0)^2}L.$$

Using the above relation, the compressibility of tubes with varying $D_0$ is calculated and matched with experimental and simulated results. The resulting turgor pressure $T$ prediction is shown in Fig. 4F.

## References


1 O. Kedem and A. Katchalsky, *Biochimica et Biophysica Acta*, 1958, **27**, 229–246.

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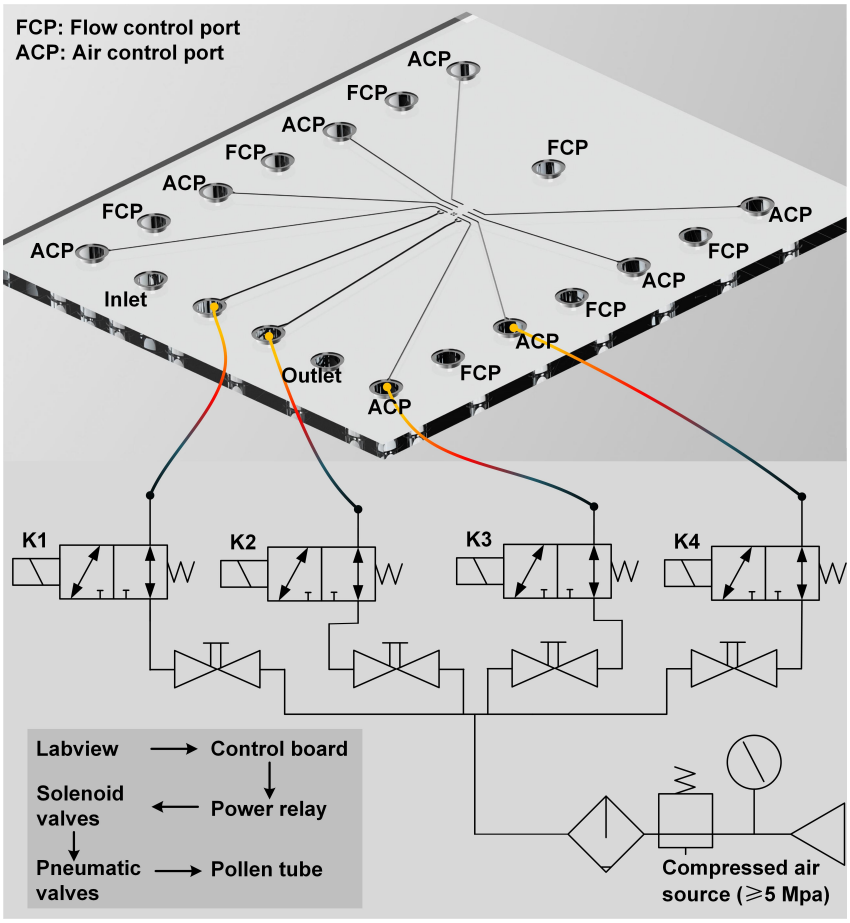



**Fig. S1** Automatic pneumatic control system for microvalve pressurization. The inlet shows the schematic of pneumatic control.

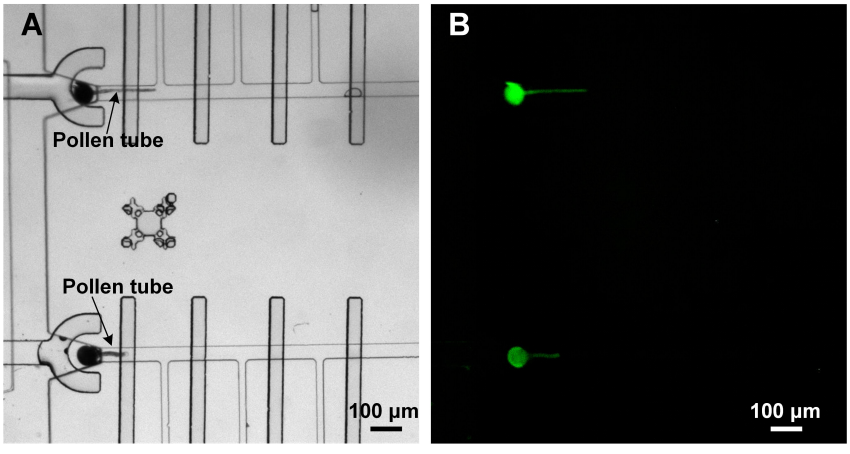



**Fig. S2** Two pollen tubes are trapped simultaneously in the LOC device. (A) Bright field image and (B) Fluorescent image.

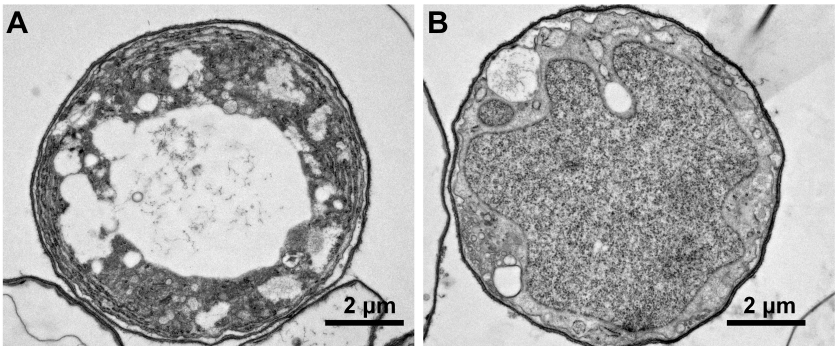



**Fig. S3** TEM image of lily pollen tube. A and B are taken at different cross sections of the pollen tube.

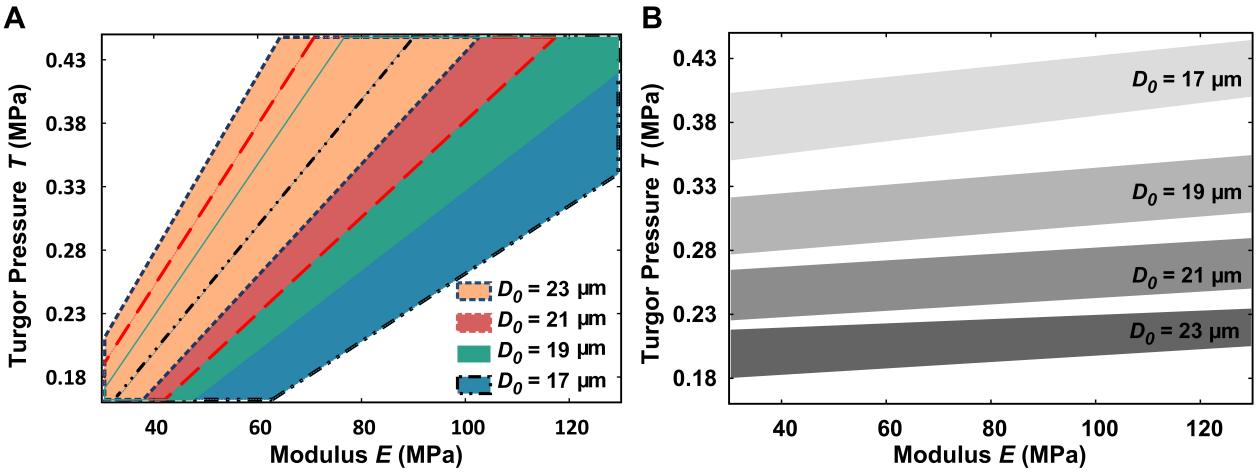



**Fig. S4** Parameter space for stretch ratio and compressibility. (A) The subspaces of simulated tube properties that fall within the 95% confidence interval of the experimental $\lambda$. (B) Simulated tube property subspaces that fall within the 95% confidence interval of the experimental compressibility.

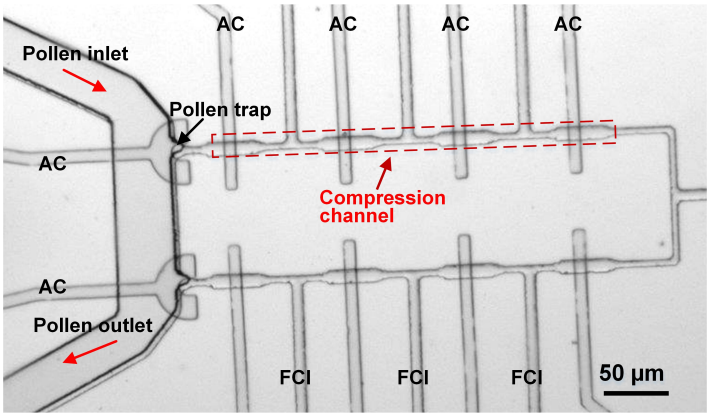
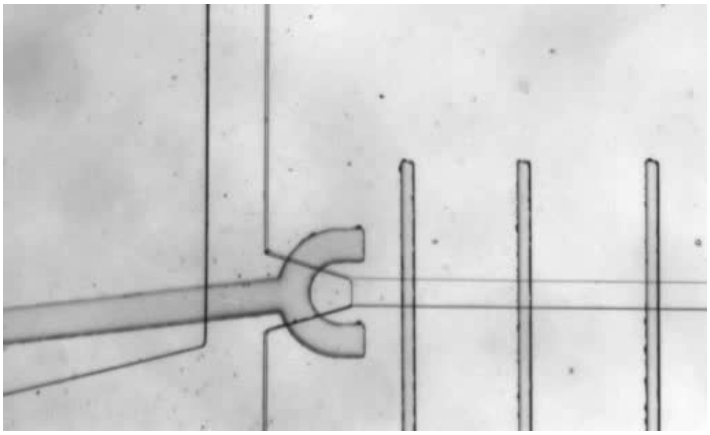
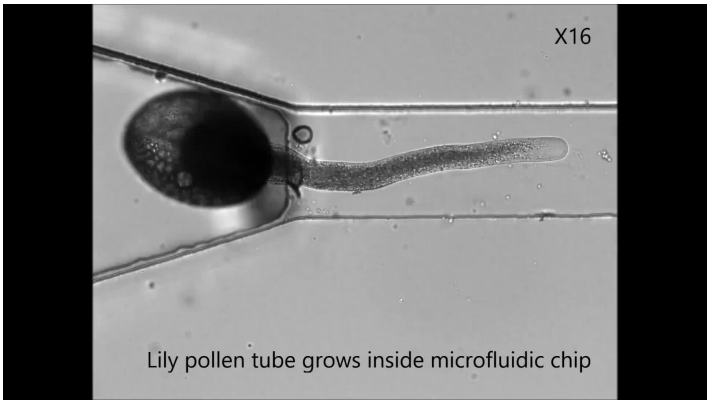


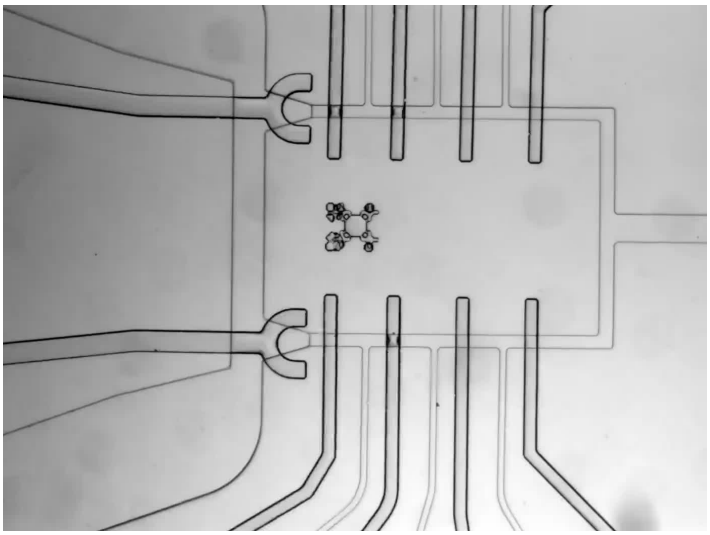

**Fig. S5** The proposed LOC device is adapted for *Arabidopsis thaliana* pollen tubes.



**Movie S1** Real time trapping of a lily pollen tube inside the LOC device. This video demonstrates the process of trapping a single pollen tube by reorientating the pollen tube by the flow inside the microchannel and fixing the pollen tube by a microvalve.




**Movie S2** Lily pollen tube grows inside the LOC device. This video demonstrates the pollen tube can grows well inside the microfluidic channel.



**Movie S3** Automatic pneumatic control of PDMS microvalves for trapping and indenting pollen tubes. This video demonstrates the independent control of PDMS microvalves by the automatic pneumatic control system. The applied pressure can also be regulated.