High precision, localized proton gradients and fluxes generated by a microelectrode device induce differential growth behaviors of pollen tubes†

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Microelectrode device fabrication

The device design was implemented on a glass substrate that provides the advantage of being transparent for optical analysis. The fabrication procedure consisted of the following steps: substrate preparation, microelectrode array deposition, insulation layer deposition, counter electrode deposition, and wiring of the electrode connection pads (Fig. S1A).

The lift-off process was employed to fabricate the microelectrode array layer, insulation layer, and counter electrode layer (Fig. S1B). The masks were designed using AutoCAD and printed on a 50800 dpi film by Selba S.A. For the photolithography process, the pre-cleaned glass wafer was dehydrated by heating it on a hot plate at 180 °C for 10 minutes. Then the wafer was naturally cooled to room temperature. AZ 9260 was spin-coated onto the wafer with a target thickness of 6 μm. In order to evaporate solvents from the resist, the wafer was left at room temperature for 5 minutes and subsequently softbaked at 110 °C for 3 minutes. Afterwards, the wafer was left to cool and rehydrate at room temperature for 10 minutes. The exposure was carried out with a Karl Süess MA6 mask aligner. The exposure dose was 900 mJ/cm^2. After the exposure, the wafer was developed for approximately 90 s in an AZ 400 K developer solution with a 1:4 ratio of developer to deionized water. To completely stop the development, the wafer was washed in a quick dump rinser until the electrical resistance of the water reached 13.5 ohm/cm. Then the wafer was dried in a wafer dryer. A 60 nm indium tin oxide (ITO) layer was deposited using a Univex 500 electron-beam evaporation system on the AZ-coated wafer. After the ITO deposition and dissolving the AZ, the wafer was annealed at 500 °C for 10 minutes to allow the ITO to be a transparent electrode. Similarly, a passivation layer of Si_3N_4 was deposited with a target thickness of 400 nm after lithography. Another lift-off process was performed to deposit the counter electrode with a 10 nm of Ti and 50 nm of Pt deposited by plasma-enhanced chemical vapor deposition (PECVD) after the lithography. Insulated copper wires were glued to the electrode connection pads using a conductive epoxy (CW2400, Circuitworks).

Oxygen effects on pollen tube growth

During the experiment, we did not find oxygen bubbles generated on the microelectrode during the experiment, the oxygen produced by water electrolysis should have been dissolved in the growth medium. In the experiment, we prepared an oxygen super-saturated lily growth medium by pumping pure oxygen into a growth medium at 5 °C, because lower temperature can increase oxygen solubility in the growth medium compared to room temperature. The growth medium was equilibrated to room temperature before use.

A microfluidic device developed previously was used for studying the oxygen effect on pollen tube growth (Fig. S6A). The microfluidic chip can trap pollen tubes in the microfluidic channel, and the oxygenated growth medium can be supplied from the side channels. The details relating to the design and fabrication of the microfluidic device can be found in a previous paper.[1] The lily pollen grains were prepared with the protocol explained in "Pollen collection and germination" and germinated in the normal growth medium. After germination, the pollen tubes were guided to the microfluidic channel and trapped, as shown in Fig. S6B and C. Then, the oxygenated growth medium was injected from the side channel with a flow rate of 50 μL/min. Pollen tube growth was recorded. From the experiment, all the pollen tubes (n=13) did not show any change of growth behavior (bursting, growth arrest, bending).

References

1 C. Hu, G. Munglani, H. Vogler, T. Ndinyanka Fabrice, N. Shamsudhin, F. K. Wittel, C. Ringli, U. Grossniklaus, H. J. Herrmann and B. J. Nelson, *Lab Chip*, 2017, DOI: 10.1039/C6LC01145D.

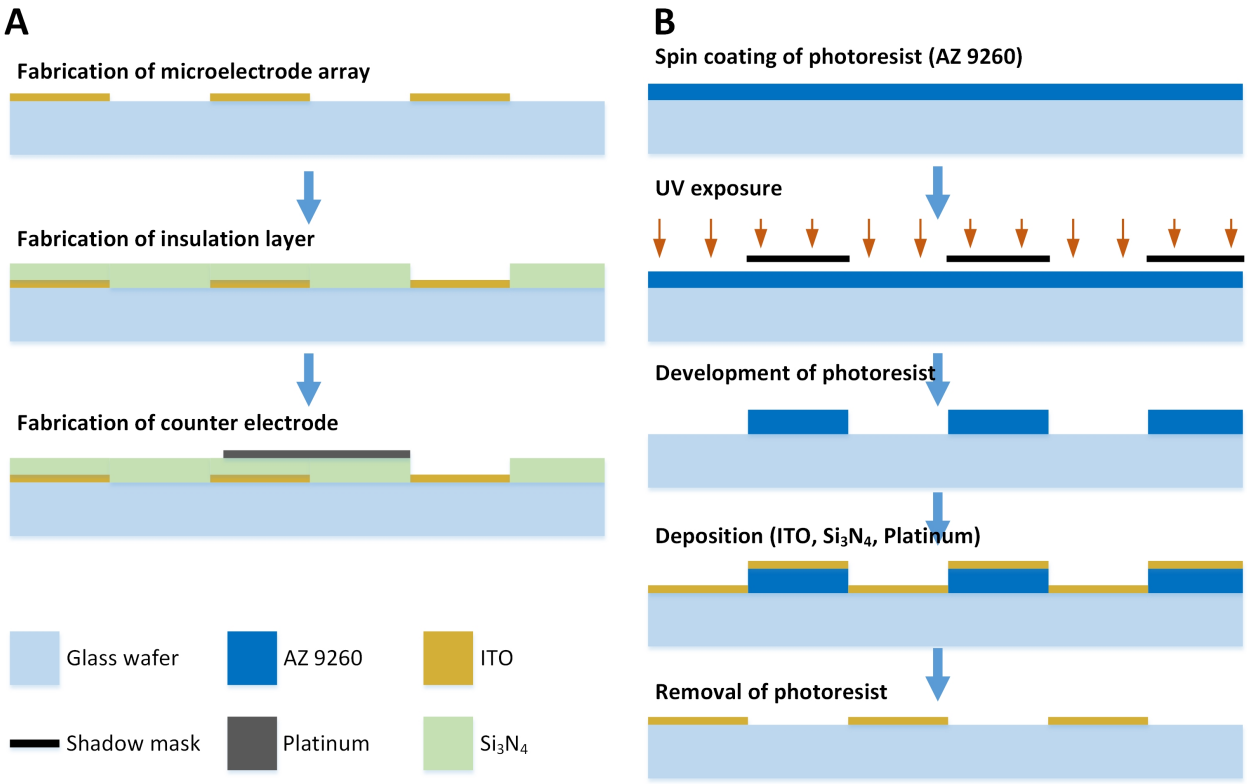


Fig. S1 (A) Fabrication process of the microelectrode device. (B) The lift-off process. A pattern is firstly defined on the substrate using photoresist and standard photolithography. Then the target material (ITO, Si_3N_4, Platinum) is deposited all over the substrate, covering the photoresist and areas in which the photoresist has been cleared. After the removal of photoresist, the deposited film is left on the substrate.

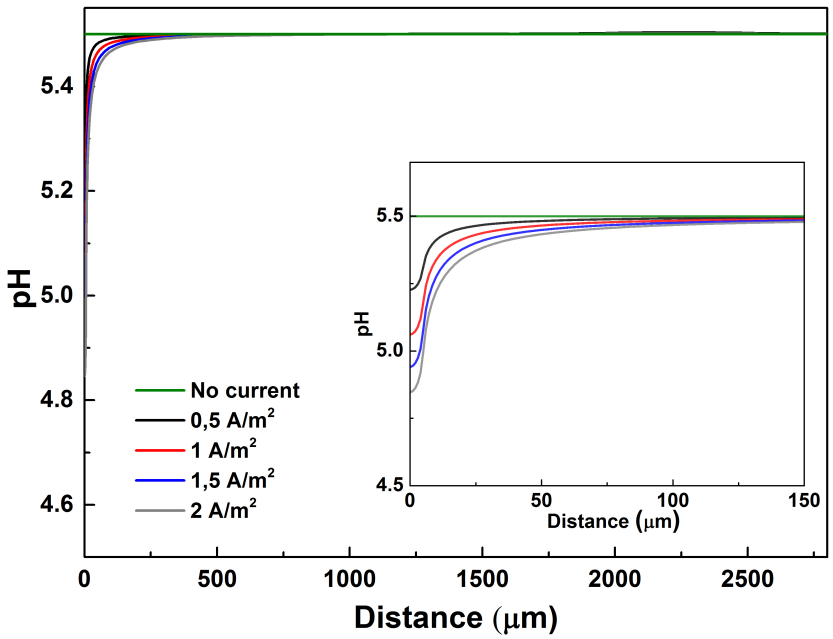


Fig. S2 The pH profile near a 10 μm wide electrode at different input current densities. The anode is located at 0-5 μm and the cathode is located at 2000-2500 μm.

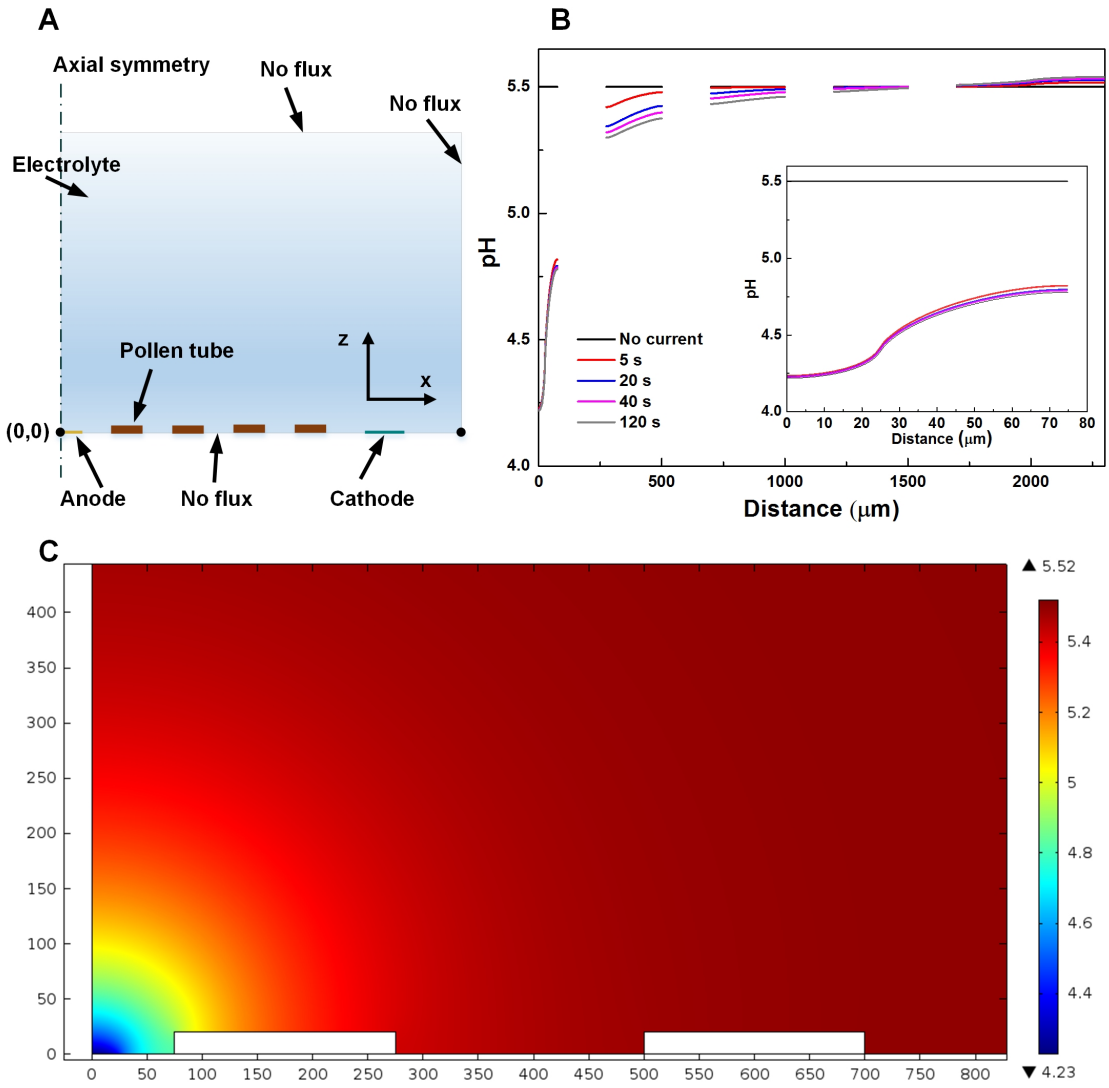


Fig. S3 Numerical simulation of the pH gradient near microelectrode due to water electrolysis when there are pollen tubes lying between anode and cathode. In the simulation, there are four pollen tubes lying between the anode and cathode, represented by four rectangles. Each pollen tube has a length of 200 μm and a diameter of 20 μm. The left bottom corner of these four rectangles start at 75 μm, 500 μm, 1000 μm, 1500 μm along the *x* axis, respectively. (A) shows the simplified 2D axisymmetric model for numerical modeling. Four pollen tubes lying along the *x* direction between anode and cathode. (B) The pH profile near a 50 μm wide anode for a current density of 2 A/m^2 at different times. The simulation was conducted for the first 120 s and the initial pH of the culture medium is 5.5. The anode is located at 0-25 μm and the cathode is located at 2000-2500 μm. (C) Colormap plot of the simulated pH distribution around a microelectrode at the steady state for a current density of 2 A/m^2 at 20 s. The anode is located at 0-25 μm.

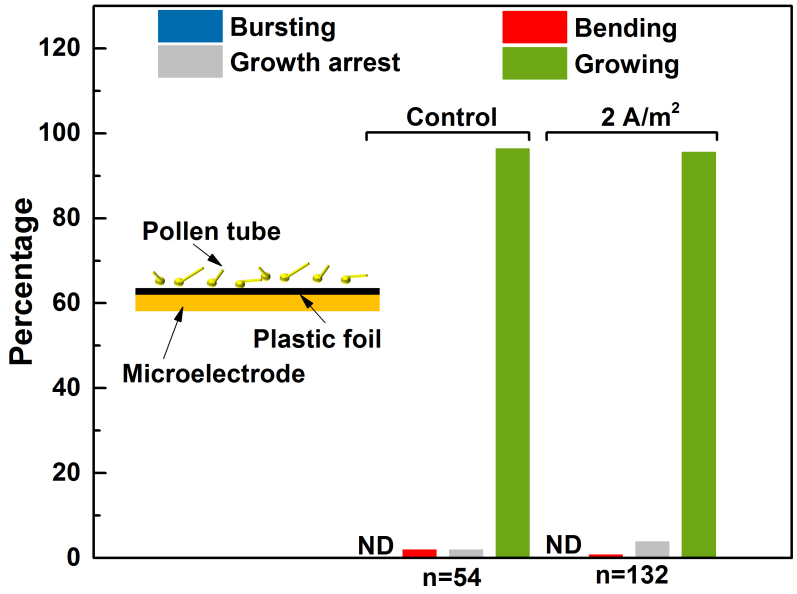


Fig. S4 The pollen tube growth behavior before and after the shielding test. The electric field has no effect on pollen tube growth when the electrodes are covered with an insulating foil. ND: No data.

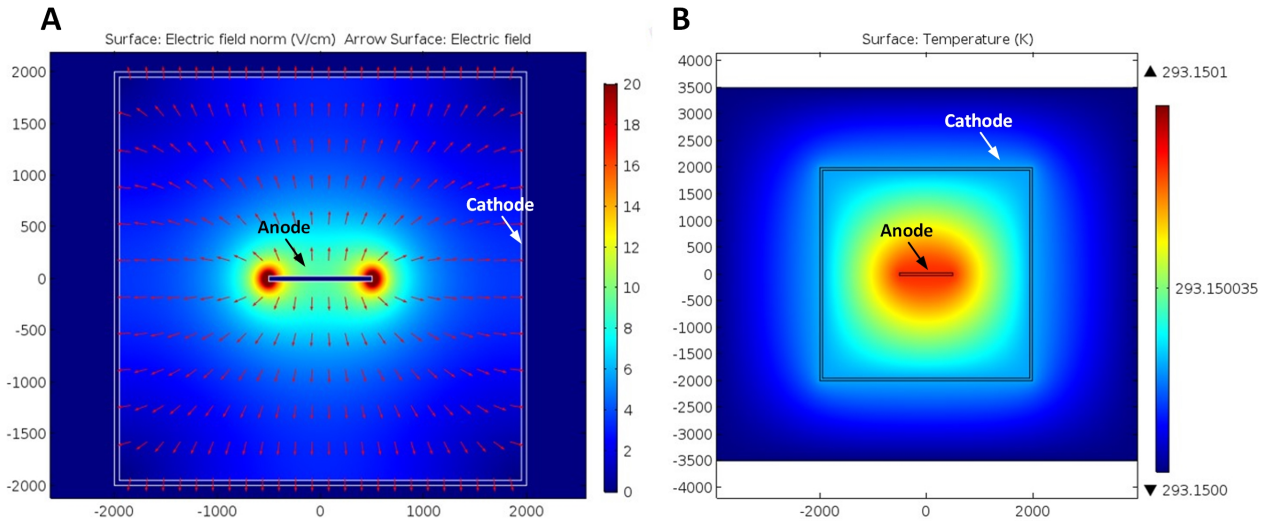


Fig. S5 (A) Simulated electric field near the electrodes for a constant current density of 2 A/m^2. (B) Joule heating generated by the microelectrode for a constant current density of 2 A/m^2.

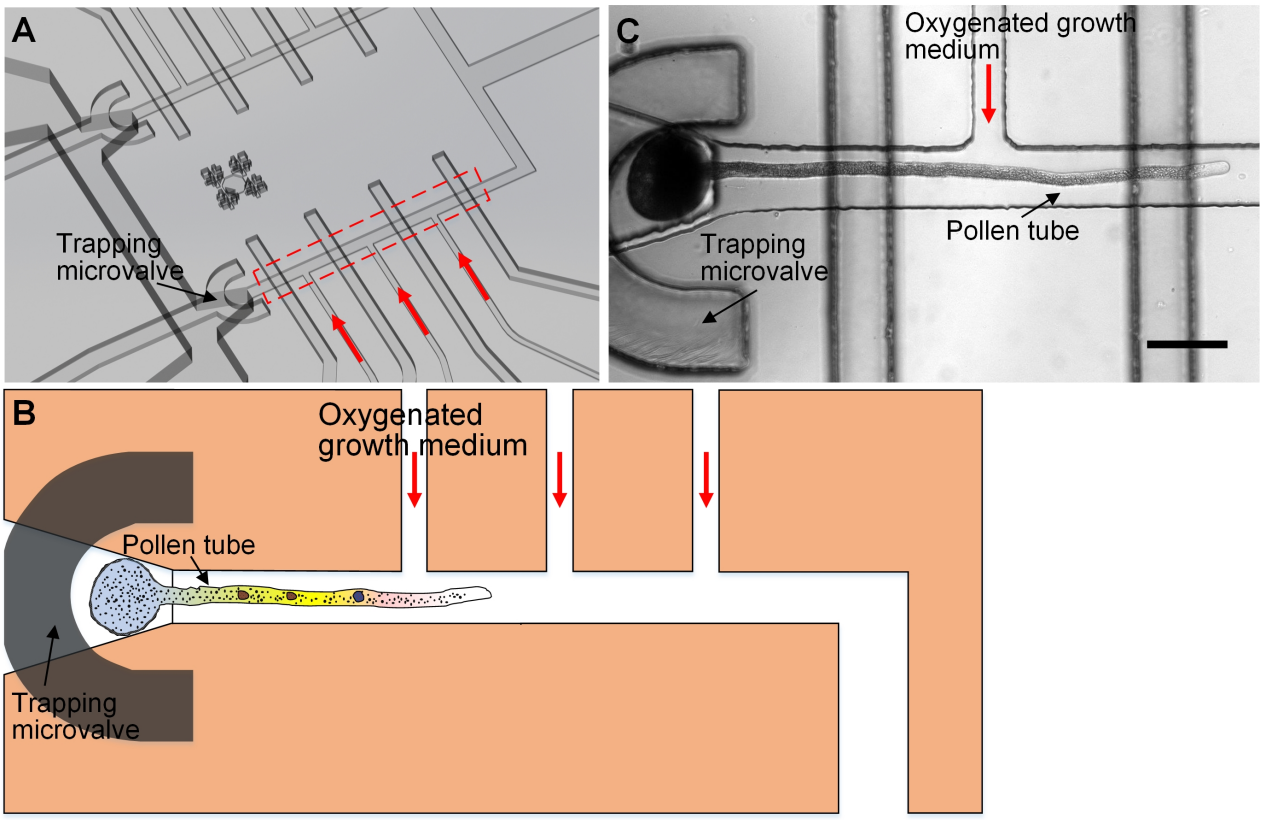
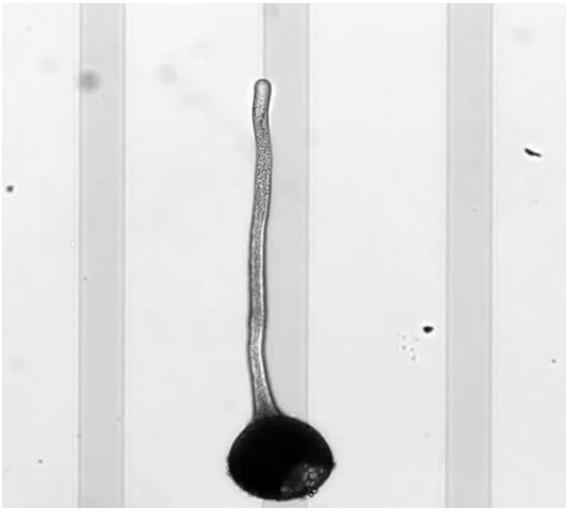
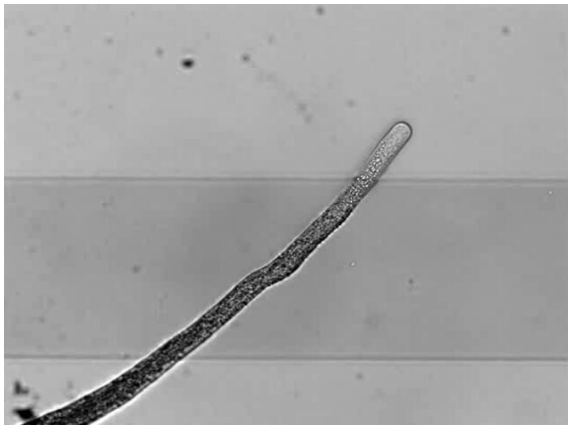


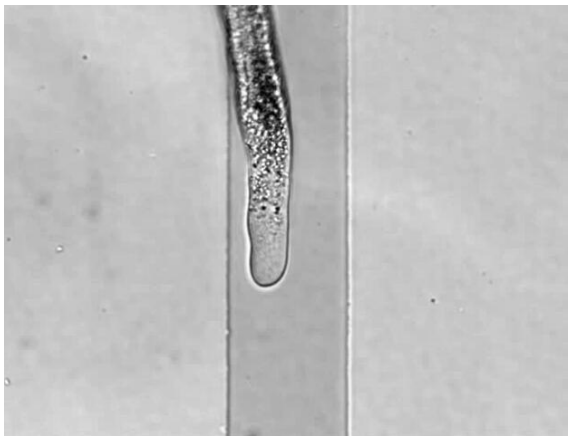
Fig. S6 Experiment of the oxygenated growth medium on pollen tube growth. (A) Top view of the LOC device. Pollen tube can be trapped by the trapping microvalve and grows along the microchannel indicated by the red dash box. The oxygenated growth medium can be injected from the side channel indicated by the red arrow. The chip enables the trapping of two pollen tubes simultaneously. (B) illustrates the working principle of the device: a pollen tube is trapped inside the microfluidic channel; 3 side channels provide the oxygenated growth medium to the trapped growing pollen tube. (C) Bright field image of a pollen tube that is trapped in the microfluidic channel and grows along the channel. Scale bar: 100 μm.



Movie S1 Pollen tube bursting near an electrode. The time scale in this movie has been compressed by a factor of two.



Movie S2 Pollen tube bending near an electrode. The time scale in this movie has been compressed by a factor of 30.



Movie S3 Pollen tube stops growing on top of an electrode. The time scale in this movie has been compressed by a factor of 12.