**SUPPLEMENTARY MATERIAL**

# Determination of the temperature-dependent cell membrane permeabilities using microfluidics with integrated flow and temperature control

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## Supplementary Movies

**Movie S1** Shows cell volume historical during media switching from 1x PBS to 3x PBS at room temperature, 30 ° and 37 °. This clip was captured at 30 fps for 60 secs and plays at 60 fps. Scale bar: 20 $\mu m$)
**Movie S2** Shows cell volume historical during media switching from 1x PBS to 10% (v/v) DMSO at room temperature, 30 ° and 37 °. This clip was captured at 30 fps for 180 secs and plays at 180 fps. Scale bar: 20 $\mu m$)

## Supplementary Text: Integrated microfluidic platform setup

### Microfabrication

We use standard multilayered soft-lithography (MSL) protocols for fabrication the microfluidic channel and the wet etching process for conductive wire micropatterning (see Result section). The mask design is also available as a separate file in ESI.

### Conductive wire patterning

A glass slide (EMF, CA-134) with a 1000 Å thick gold film on a 50 Å thick chrome adhesion layer was used for conductive wire patterning. The slide was spincoated with a positive photoresist with thickness $\sim$ 1.5 $\mu$m (Microchem, AZ-1512), followed by a soft baking process at 110 °C for 2 min. The microheater and the adjacent temperature sensor were designed by AutoCAD and printed on a transparency with 50,800 dpi resolution (Fineline Imaging, OR). The exposure process was carried out by using a mask aligner (ABM-aligner, ABM-USA Inc.) under wavelength of 365 $\mu$m for 5–6 sec. The photoresist was then developed by using 25 % v/v aqueous photoresist solution (Microchem, AZ-340) for 1 min. The glass slide with the patterned photoresist was baked at 110 °C for 1 hr for better adhesion and curing. A wet etching process was conducted by immersing the glass slide into gold etchant (TFA gold etchant, Transene) for 30–35 sec, followed by immersing the glass slide immediately in chrome etchant (TFE chromium etchant, Transene) for 2–3 sec. The glass slide was rinsed by acetone to remove residual photoresist. The etched glass slide was again rinsed with isopropanol (IPA) and water, and dried by nitrogen gas. The glass slide, together with the electric connection pads covered by Teflon tape, was coated with 100 nm silicon dioxide to prevent any electrolysis.

### Double layered microchannel fabrication

The microfluidic channel was fabricated using standard multilayer soft-lithography (MSL). Photomasks were designed using AutoCAD and printed on a transparency with 50,800 dpi resolution using commercial laser photoplotting service (Fineline Imaging, OR). The masters were fabricated by contact photolithography using a negative photoresist (SU-8 2050, Microchem). The photoresist was spincoated onto a plasma-cleaned 100 mm silicon wafer (test grade wafer, SVM). After a soft baking process at 65 °C for 3 min then at 95 °C for 9 min, an exposure process was carried out by using a mask aligner (ABM-USA Inc., ABM-aligner) with a 365 $\mu$m wavelength UV light for 22 sec. After post baking (2 min at 65 °C, 7 min at 95 °C), the master was then developed with a propylene glycol monomethyl ether acetate (PGMEA) (Y020100, Microchem), followed by surface treatment with trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (448931, Sigma-Aldrich) vapor under a vacuum condition for easy peeling of PDMS replica from the master.
Two SU-8 masters of fluidic layer (70 $\mu$m deep) and control layer (70 $\mu$m deep) were fabricated. The fluid layer with a thin PDMS valve membrane was obtained by spincoating the fluidic SU-8 master with PDMS (Sylgard 184, Dow Corning) at 15:1 ratio of the base resin and the crosslinker. The control layer was fabricated by casting PDMS at 5:1 ratio of base resin and crosslinker onto the corresponding SU-8 master. Both PDMS layers were partially cured by baking at 65 °C for 25 min. The thick PDMS replica (control layer) was then peeled off from the master, aligned, and was hermetically sealed onto the thin PDMS layer (fluidic layer). The bonded PDMS double-layer was placed on a hot plate at 65 °C for 4 hr to form an integrated monolithic device.

A step-by-step fabrication protocol for the device fabrication is provided as below.

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**Master making process: using SU-8 2050**

- Turn on hot plates, UV power, and spinner
- Clean silicon wafer(s), rinse with
  - Acetone-IPA-Water-IPA
  - Dry with $N_2$
- Place wafer on hot plate at first setting, for few minutes to remove excess solvent
- Remove wafer and let cool, then pour SU-8 2050 negative photoresist ($\sim$2mL/inch substrate diameter).
- To prevent edge effects, rotate wafer with tweezers to ensure a uniform distribution of photoresist
- Place the wafer on the spin coater and follow the recipe below:

**Control layer:** 70 um (SU-8 2050)

| | RPM | RPM/S | DWELL (s) |
|---|---|---|---|
| 0 | 1 | 100 | 1 |
| 1 | 500 | 100 | 5 |
| 2 | 2000 | 300 | 30 |
| 3 | 0 | 200 | 0 |

**Fluidic layer:** 70 um (SU-8-2050)

| | RPM | RPM/S | DWELL (s) |
|---|---|---|---|
| 0 | 1 | 100 | 1 |
| 1 | 500 | 100 | 5 |
| 2 | 2000 | 300 | 30 |
| 3 | 0 | 200 | 0 |

- Start UV Lamp and **soft bake** the wafer on the hot plate and cover with glass dish for:

| | 65°C (min) | 95°C (min) |
|---|---|---|
| 70um | 3 | 9 |

- Let wafer rest on bench for $\sim$10 min in open petri dish
- Apply mask and expose to UV for 22 s at 9.5mW/cm$^2$
- Delay 5-10 min after exposure before developing.
- **Post bake** wafer on hotplate and cover with glass dish for:

| | 65°C (min) | 95°C (min) |
|---|---|---|
| 70um | 2 | 7 |

- Use developer PGMEA for 6-7 mins until clean, remove hazing by rinsing with developer then IPA, repeat if necessary.
- Let wafer cool then rinse with IPA and gently dry with $N_2$
- Silanize wafer with trichlorosilane for 10–20 min in a vacuum desiccator.

**PDMS process:**

- Pour and mix PDMS 15:1 and 5:1 of base : crosslinker).
- Place both cups in desiccator and hold vacuum for at least $\sim$ 10-15 min. Remove bubbles by shaking against soft tubing.
- Pour small amount of 5:1 PDMS on top control layer master (first time around 80g in total, the rest 24g).
- Pour 15:1 PDMS on top of fluidic layer master.
- Then vacuum desiccate both again for 5 min.
- Then spin coat 15:1 PDMS/fluidic layer master for:

| | RPM | RPM/S | DWELL (s) |
|---|---|---|---|
| 0 | 1 | 100 | 1 |
| 1 | 500 | 200 | 5 |
| 2 | 900 | 300 | 30 |
| 3 | 0 | 100 | 0 |

- Then spin coat 5:1 PDMS/control layer master for:

| | RPM | RPM/S | DWELL (s) |
|---|---|---|---|
| 0 | 1 | 100 | 1 |
| 1 | 500 | 100 | 5 |
| 2 | 500 | 0 | 30 |
| 3 | 0 | 100 | 0 |

- Pour remaining 5:1 PDMS onto control layer.
- Then place both masters/PDMS into 65 °C oven for 25-30 min.
- Remove both masters/PDMS and let cool.
- Once cooled down remove control layer PDMS slab with scalpel and punch valve ports.
- Position the control layer PDMS slab to line up with the fluidic layer master/PDMS.
- Remove air bubbles by compression of flat tool.
- Place fluidic layer master/PDMS/Control layer PDMS slab into 65 °C oven for 1 hour - overnight.

**Microheater & sensor patterning**

Gold coated slides: EMF CA134 (Cr 50 Å, Au 1000 Å, sized at 1" x 3" x .040")

- Clean slide (s), rinse with
  - Acetone - IPA - Water - IPA
- Dry with $N_2$
- 110 °C dry solvent
- Place and double tape slide on spin coater and follow recipe: (AZ 1512)

| | RMP | ACC | DWELL (s) |
|---|---|---|---|
| 0 | 1 | 1 | 1 |
| 1 | 500 | 100 | 5 |
| 2 | 3000 | 400 | 30 |
| 3 | 0 | 500 | 1 |

- **Soft bake** 110 °C for 2 mins before UV exposure.
- Make sure by microscope that the sharp face of the transparency should be faced to gold slide directly, exposure 5-6s.
- 4:1 ratio of water : AZ 340 for 1mins, after develop, put slides into water and rinse.
- **Post bake** 110 °C for 1 hour.
- Wet etching:
  - Gold etching: 30-35s
  - Chrome etching: 1-2s
  - Water rinsing
- Put patterned slides into acetone for 2 hours
- Rinse slide(s) with
  - IPA - Water - IPA
  - Dry with $N_2$

**Deposit silicon oxide layer to slides:**

- Clean patterned slide (s), rinse with
  - Acetone - IPA - Water - IPA
  - Dry with $N_2$
- Cover the connection pads with Teflon types.
- Mount slides to sample holder and place to deposition chamber.
- Deposit silicon oxide layer.
- Peel off the Teflon tapes.

**Bonding:**

- Remove fluidic layer master/PDMS/Control layer PDMS slab from oven and let cool.
- Use scalpel to cut along the control PDMS edge to remove both layers.
- Chop excess PDMS edges with razor blade, separate each device, and hole punch inlet ports in the fluidic layer.
- Clean PDMS with tape and clean patterned slide(s) with deionized (DI) $H_2O$. Then rinse with IPA.
- Dry patterned slide(s) with $N_2$ and place on metal plate sample holder next to PDMS slab that sits side up
- Place into the chamber of plasma cleaner (Harrick Plasma, PDC-32G), set the RF level to MED and after reaching between 500 mTorr -1Torr run for 90 s allowing a small amount of air into the chamber every 30 s.
- When time is up, remove and immediately bond to the patterned slide by placing one edge at desired location and dropping the slab to force air out, then put the bonded device on the hot plate (65 °C) baking for 1 hour –overnight.

## Supplementary Text and Figures: Temperature sensor calibration

The underlying mechanism of temperature measurement in the temperature sensor rests upon the temperature dependency of the electrical conductivity of the wire. Thus, it is important to calibrate the temperature sensor to obtain a reliable and precise temperature.

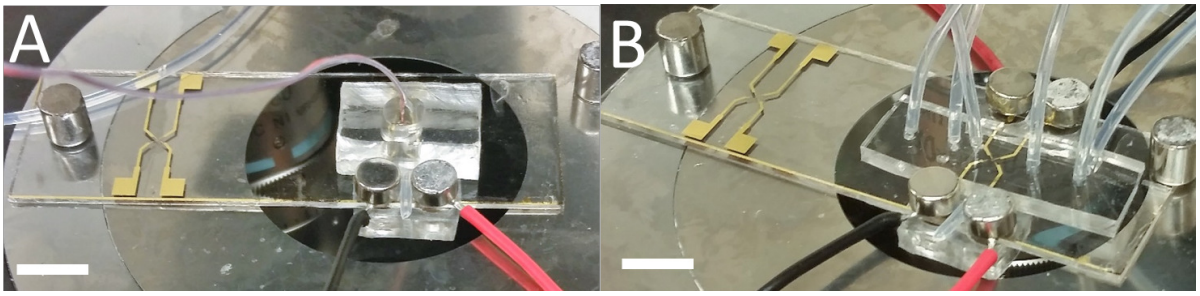


**Fig. 1** Snap shots of the microfluidic device setup (scale bar = 10 mm) and temperature control performance evaluation for (a) Setup for Temperature sensor calibration. The temperature sensor was connected to one channel of the digital multimeter while J-type thermocouple was connected to the other channel of the digital multimeter; (b) Double-layered microfluidic device with inlet and outlet tubings rest onto an inverted microscope. Wires from the microheater and temperature sensor were connected to the temperature controller and the multimeter respectively.

A PDMS slab with a reservoir, holding calibration fluid, was placed on the glass slide etched with the microheater and temperature sensor. A J-type thermocouple (Omega, SA1-J) was placed into the reservoir as the temperature reference. The temperature sensor was connected to one channel of the digital multimeter for 4-wire resistance measurement, while the thermocouple was connected to the other channel for the temperature reading (**Fig. 1a**). The reservoir was then filled with hot water, the calibration fluid, while measuring both the temperature and sensor resistance simultaneously. A series of resistance data which corresponds to the temperature ranging from room temperature to 60 °C was collected to obtain a linear curve fit that was used as a general calibration curve between the sensor resistance and real temperature. This calibration curve was implemented into the temperature control algorithm in the LabVIEW code. Once the calibration is finished, the PDMS slab was removed from the glass slide, and the double-layered microfluidic channel was bonded with the glass slide. Inlet and outlet tubings were then connected to the device (**Fig. 1b**) for further experiments.

## Supplementary Figures: Multiphysics simulation details

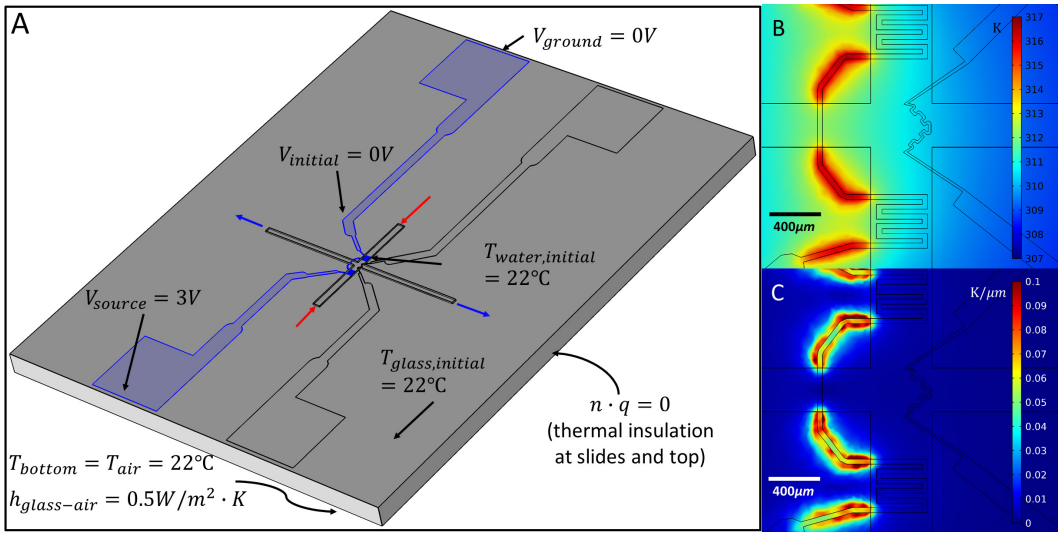


**Fig. 2** More details about multiphysics simulation: (A) Boundary and initial conditions of multiphysics simulation, where the part marked as blue indicates the wire of microheater, the red arrows implies the flow inlets and the blue arrows implies the flow outlets. The dimensions of simulation geometry are identical to designed device; (B) plane temperature distribution on the top surface of glass and (C) plane temperature gradient distributions on the top surface of glass obtained by numerical simulations.

The numerical simulation involves electric currents, heat transfer and laminar flow, with the coupling effect of boundary electro-

magnetic heat source and non-isothermal flow. The details about the boundary and initial conditions are showed in Fig. 2A. Fig. 2B and C demonstrate the plane temperature profile on the top surface of glass.

## Supplementary Figures: Cell image analysis

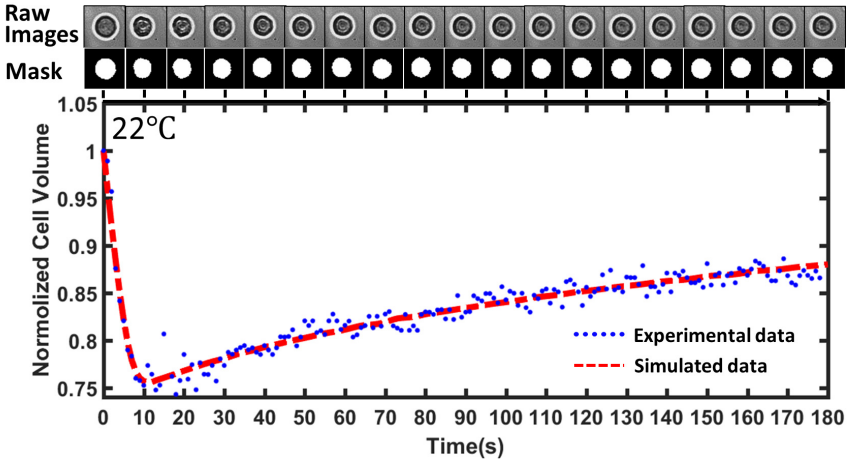


**Fig. 3** Cell volume excursion under 10% (v/v) DMSO perfusing at 22°C. The result raw images before image processing and the segmented cell masks are also showed above the analysis result plotting.

## Supplementary Table: The osmolalities of perfusion solutions used in experiment

**Table 1** The osmolalities of perfusion solutions used in experiment

| Perfusion solutions | Osmolality (mOsm/kg-$H_2O$) |
|---|---|
| 0.7× PBS | 212 |
| 1× PBS | 300 |
| 1.5× PBS | 426 |
| 2× PBS | 598 |
| 3× PBS | 839 |
| 10% (v/v) DMSO in 0.9% NaCl | 1846 |

## Supplementary Text and Figures: Sphericity ananlysis of processed cell images

To evaluate the how well the sphere cell shape assumption is across the cell volume changing, we involves the sphericity, which was defined as $2\pi \cdot r_{equ}/p_{act}$. Here, $r_{equ}$ is the equivalent cell radius calculated with the two-dimensional cell area based on spherical cell shape assumption, and $p_{act}$ is the perimeter of the obtained two-dimensional mask of trapped cell. As showed in Fig. 4, the cell sphericities excursion during perfusion by hypertionic solutions are generally above 0.9, at some worst cases it reaches around 0.7, which indicates that, for Jurkat cells, the spherical cell shape assumption fits the actual experiment situation fairly well.

## Supplementary Text and Figures: LabVIEW program

### Hardware components

All the hardware components were real time controlled with a custom made LabVIEW program. The hardware is not limited to specific build up. Components from other venders will also work after proper modification of hardware Input/Output (I/O) setup in LabVIEW.

### Real time image processing

The LabVIEW built-in Vision Assistant Express module was used for image processing, involving the feature enhancement and particle analysis. The real time image processing for cell segmentation is challenging, as the illumination condition of cell change dynamically.

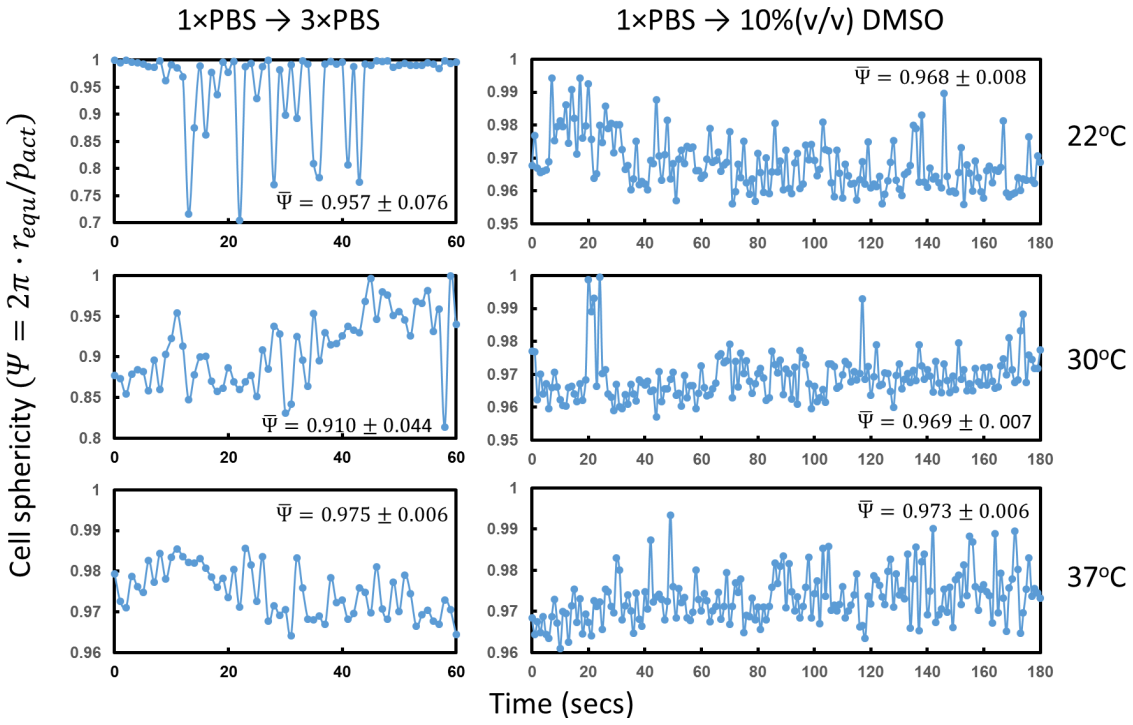


**Fig. 4** The cell sphericity excursion during perfusion by hypertonic solutions at various temperature: The left column: 1×PBS to 3×PBS at 22°C, 30°C, 37°C; The right column: 1×PBS to 10% (v/v) DMSO at 22°C, 30°C, 37°C.

To achieve optimized results, we incorporate the following functions: threshold, thin, fill holes, removal of small objects and particle analysis. See example image processing sequence showed in **Fig. 5**, from raw images to particle detection.

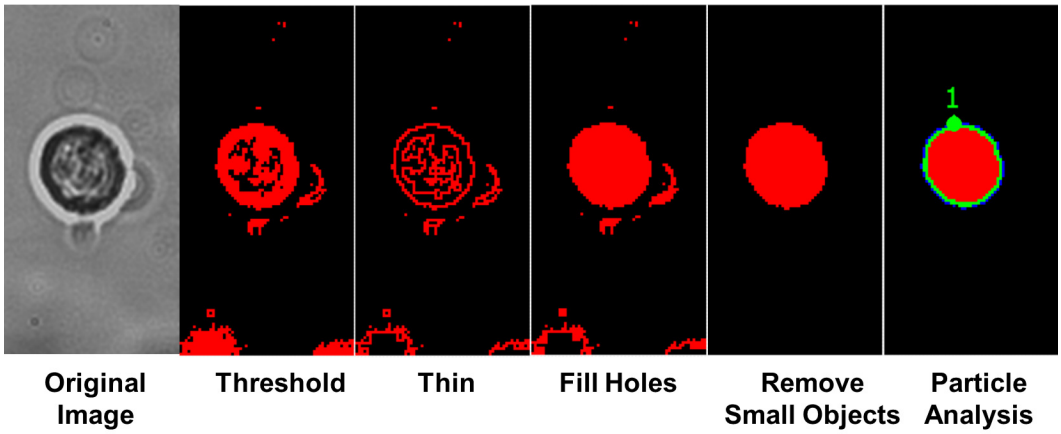


**Fig. 5** Image processing sequence: The image was first threshold to show mainly the outline of cell; Then by several steps of morphology enhancement (thin, fill holes and remove small objects), the cell feature was segmented as cell mask, the particle analysis was conducted to result cell mask and the centroid and area of cell can by obtained, which can be use for the cell trapping feedback control.