A vast-range speed control microchip for retention of all cell types

Supplementary Information

Contents

1 How to design and use speed-control chamber?

1.1 Why do we use a trumpet-shaped chamber?

A rectangular chamber is the simplest microfluidic device. When the flow-rate in the chambers is properly controlled, adherent cells are able to be retained and cultured. Fig. S1A shows the flow and Fig.S1C shows the distribution of flow-rates within a rectangular chamber. The flow-rates in the rectangular chamber are distributed very evenly, except very near the walls. Changing the flow-rate within a rectangular chamber leads to a new, but equal, flow-rate at each corner and, therefore, if the flow-rate at one site is not as required, it will not be suitable anywhere within the chamber.

Fig.S1B shows a trumpet-shaped chamber. When the flow through the chamber is from left to right, the flow slows gradually because of the increasing diameter of the chamber. This chamber is a flow-rate gradient-generating microfluidic device. The expected distribution of flow-rate along a speed-control chamber is illustrated by Fig.S1D. The required flow-rates are still within the new range (see the broken line in Fig. S1D). On the other hand, the flow-rate is reduced significantly with increasing diameter of the chamber, and any cell of very low adhesion ability and high suspensibility is retained in the chamber. Cells spread and separate from each other in the flow field (see Fig.S1B). Any microdevice can be embedded into the speed-control chamber (Fig.S1E and F) to help the chamber retain cells or to help a complex design that requires a certain flow field (correct flow-rate and orientation). Micro-devices can be arranged as shown in Fig.S1E and F (compare the flow field in Fig.S1B) to ensure the required flow-rate and orientation. We tested the simple design shown in Fig.S1F, with arched speed bumps embedded in the open area to aid the function of the speed-control chamber.

1.2 Why do we use exponential shape?

The predictability and controllability determine the quality of the chamber. A simple triangular chamber reduces flow-rate significantly, but the distribution of flow-rates is unsatisfactory because only the area near the input is effective (but too rapid). In the middle and at the end of the chamber, the reduction of velocity is very slow and is linear rather than logarithmic or exponential. Logarithmic or exponential functions are natural functions of variables such as loudness, brightness or even the size of cells; exponential widths, not linear widths, are even and the whole chamber is effective. A logarithmic or exponential function enhances the predictability and controllability of a speed-control chamber; at any point in the speed-control chamber, a fixed distance increment from the input results in a deceleration by half. If an adjustment of the driving pressure doubles the chamber's average velocity, any previous velocity can be found at a fixed distance downstream.

1.3 How to calculate the equal interval bumps?

If the width is W_0 (see Fig.S1G) at the entrance (x = 0), then each distance D results in deceleration by a half. The width W_x at x is:

$$W_x = W_0 \, 2^{x/D} \quad (1)$$

Let $D' = D/\ln 2$, we have:

$$W_x = W_0 \, e^{x/D} \quad (2)$$

The trim size is determined by the input width W_0, output width W_e and the length of the chamber, L. The internal parameters D and D' are calculated by

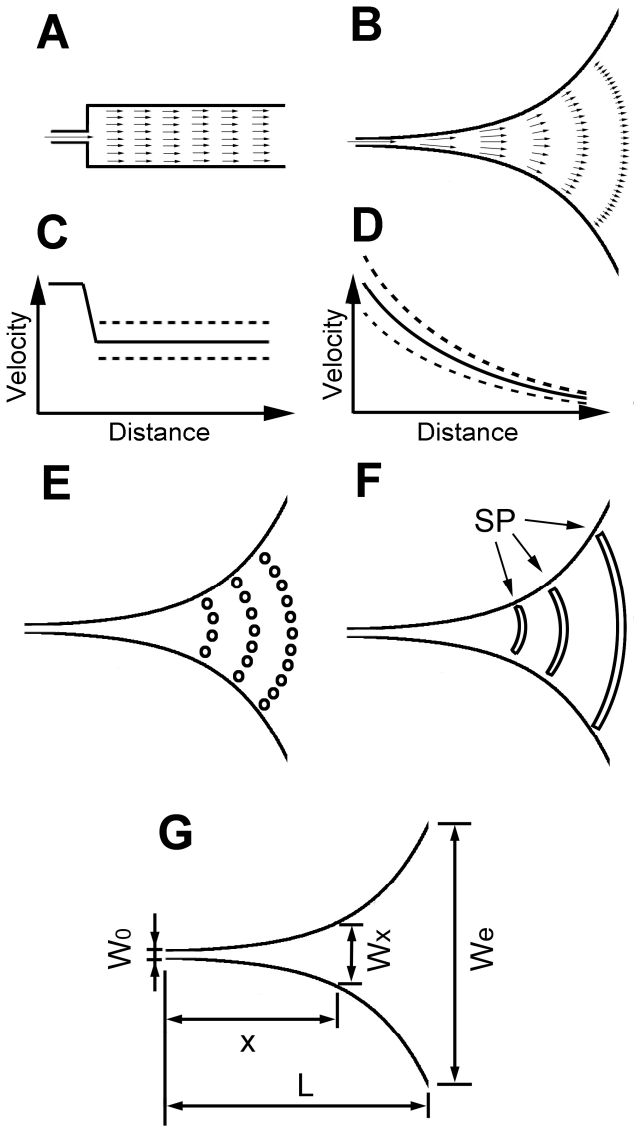

Fig.S1 The design concept of a speed-control chamber. **A** The flow field in a rectangular chamber (arrows show the strengths and the directions of the flow field from left to right, similarly hereinafter); **B** The flow field in a speed-control chamber; **C** The velocity distributions of the flow in the rectangular chamber (**A**). **D** The velocity distributions of the flow in the speed-control chamber (**B**). The dash lines in **C** and **D** represent fluctuation patterns of the velocity distributions. The patterns of array (**E**) or dams (**F**) show the ways to embed micro devices in the open area of a speed-control chamber, in order to strengthen or to add any functions for single cell cultures, measurements or other complex cell-cell experiments. The calculation principle for the shape of a chamber and its bumps are depicted in **G**. L is the length, W_0 is the starting width, W_e is the ending width and W_x is the width at x. **SP:** speed bumps.

Eqs 3 and (4), respectively:

$$D = L \ln 2 / (\ln W_e - \ln W_0) \quad (3)$$

$$D' = L / (\ln W_e - \ln W_0) \quad (4)$$

The streamlines in a speed-control chamber follow an approximate exponential curve, which has a calculable length, and are straight only along the central axis. Speed bumps are designed to follow a path of the same length as these curves; the numerical value of a speed bump's shape was calculated by a Visual Basic computer program, and the mask design was eventually drawn by this program. Eq. (5) is used to calculate the curve lengths and the shapes of the speed bumps. Let:

$K = \ln (W_e / W_0) / L$

$K_1 = \sqrt{} (1 + (W_0/4)^2 K^2 e^{2K})$

$K_2 = \sqrt{} (1 + (W_0/4)^2 K^2)$

And the length (M) at x ($0 < x < L$) is:

$$M = (K_1 - K_2 + \ln((K_1 - 1) / (K_1 + 1)) / 2 - \ln((K_2 - 1) / (K_2 + 1)) / 2) / K \quad (5)$$

1.4 VB codes for chip design demonstration

This program requires one picture box, two command buttons and four text boxes (for W_0, W_e, L and bump interval, respectively) on a form. A VB6 file "form1.frm" can be directly loaded for demonstration. This program shows how to design a speed-control chamber with bumps.

```
Private Sub Command1_Click()
plot_SCC Val(Text1), Val(Text2), Val(Text3)
End Sub
Public Sub plot_SCC(Width_start As Double, Width_end As Double,
Longness As Double)
Dim Width_start1 As Double
Dim Width_end1 As Double
Dim D As Double
    S_E_Ratio = Width_end / Width_start
    Decr_P_D = Log(S_E_Ratio) / Longness
    For i = 1 To 1000
        x = Longness * i / 1000
        y = Width_start * Exp(Decr_P_D * x)
        Picture1.PSet (x, y)
        Picture1.PSet (x, -y)
    Next i
    D = 0
    Do Until D > Longness
      For i = 1 To 500
          Width_start1 = Width_start * i / 500
          Width_end1 = Width_end * i / 500
          x = X_calculator(D, 0.1, Width_start1, Width_end1, Longness)
          y = Width_start1 * Exp(Decr_P_D * x)
          Picture1.PSet (x, y)
          Picture1.PSet (x, -y)
      Next i
      D = D + Val(Text4)
    Loop
End Sub
Private Sub Command2_Click()
Picture1.Cls
End Sub
Private Sub Form_Load()
Picture1.Scale (-200, 500)-(1000, -500)
Text1 = 10:Text2 = 400:Text3 = 800:Text4 = 10
End Sub
Private Static Function Len_calculator(a As Double, b As Double, c As
Double, D As Double) As Double
```

```
Dim K As Double, K1 As Double, K2 As Double
    K = Log(b / a) / c
    K1 = Sqr(1 + a * a * K * K * Exp(2 * D * K))
    K2 = Sqr(1 + a * a * K * K)
    Len_calculator = (K1 - K2 + Log((K1 - 1) / (K1 + 1)) / 2 - Log((K2 - 1) / (K2 + 1)) / 2) / K
End Function
Private Static Function X_calculator(Len_Given As Double, err_Given As Double, a As Double, b As Double, c As Double) As Double
Dim start_Given As Double
  start_Given = c
  Do
    Len_Err_Now = Len_Given - Len_calculator(a, b, c, start_Given)
    If Abs(Len_Err_Now) < err_Given Then Exit Do
    start_Given = start_Given + Len_Err_Now / Len_x_slope(a, b, c, start_Given)
 Loop
  X_calculator = start_Given
End Function
Private Static Function Len_x_slope(a As Double, b As Double, c As Double, D As Double) As Double
K = Log(b / a) / c
Len_x_slope = Sqr(1 + a * a * K * K * Exp(2 * D * K))
End Function
```

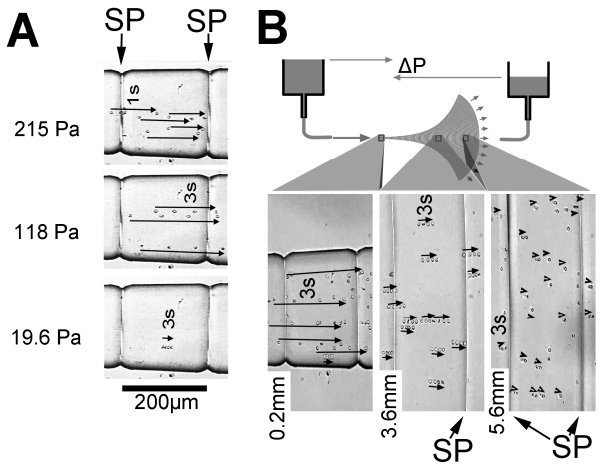

Fig.S2 The velocity distributions in a speed-control chamber and its reactions to pressure change. **A** Micro flows inside the speed-control chamber are indicated by chicken red cells. Images at intervals of 1s (0s, 1s, 2s, 3s) are superimposed together to show the movement of single cells. Arrows indicate the moving distances of single cells in 3s or 1s when flows are driven by pressure of 19.6Pa, 118Pa or 215Pa (similarly hereinafter). **B** Velocities near the entrance (left, 0.2mm from the entrance), in the middle (3.6mm form the entrance) and near the outlet (right, 5.6mm from the entrance) when a pressure difference (**ΔP**) is applied (from left to right) to the speed-control chamber (see video clip PengSI_1.avi for more detail). **SP**: speed bumps.

2 Experimental

2.1 Pressure control

The flow-rate in a micro-channel is very sensitive to pressure. To control the flow-rates precisely, the pressure at the input and at the output need to be controlled accurately. Plastic tubes connected to the vertical wells on the microchip are attached to small plastic bottles, and pressure is controlled by altering the level of liquid in these bottles. Each bottle is attached to a height controller (Anthone Elec. Ltd., Xiamen, China), which is adjusted by a computer. The precision of the height control is ± 0.05 mm, which translates to ±0.5 Pa for pressure.

2.2 Optical set-up

A box-like attemperator was attached to an inverted microscope below the light and over the object lens. A microchip was placed into the attemperator under microscopic inspection. Plastic tubes for pressure control and sampling passed through the walls of the attemperator. The air temperature in the attemperator was maintained at 37.0(± 0.1)ºC for mammalian cell cultures and 20.0(± 0.1)ºC for dinoflagellate cultures by a controller (Anthone Elec. Ltd., Xiamen, China) and a computer. The light used for dinoflagellate culture had an output of 6000 lx. Micrographs were obtained with a CCD camera and stored as AVI clips or serial images. Time, date, and temperature were printed onto the serial images.

2.3 Image processing and velocity measurment

Phase contrast microscopy was used for cell counting. When counting cells resting on the floor of the chamber rather than moving in the stream, we checked the video clips frame by frame to see if a cell was moving. We used image processing software (Adobe Photoshop) to identify both moving and stationary cells in the same image (see Fig.S3D). Two serial images of 0.1s were mixed; moving cells became bright and stationary cells became dark. More than two images were mixed to show the route of a moving cell (see Fig.S2A, B, Fig.4C) or to calculate the flow-rate of cells. One image was selected as the background and the other images were stacked above it. Cells in the upper layers were selected and the other parts of these upper layers were deleted. To highlight the selected cell, its edges were brightened or the color was changed before all layers were merged. Color images were changed to gray (Fig.S4C and D) and some cells discussed in the text were manually painted green, red, yellow or orange in order to highlight them and to dim others.

Chicken blood was collected into plastic tubes and 500 IU/ml sodium heparinate was added as an anticoagulant to make the blood flow well. The red blood cells were diluted with serum to a concentration of 1000 cells/μl and injected into the chamber under controlled pressure (as described above). The movements of cells within the chamber were recorded with a video camera (JVC TK-C921EC). At 1 min, when the pressure was stable, more than 20 cells were recorded onto a video clip at

30 frames/s. The pressure on the inlet well was then adjusted to a new value and the measurement was repeated. The image processing of moving cells described above was used to measure the distances moved during a certain length of time (1–10s). Velocities of moving cells were calculated for statistical evaluation of the velocity of the liquid flow.

2.4 Multi-cell cultures of rabbit bone marrow aspirate

Rabbit bone marrow cells were collected from the bones of domesticated rabbits. Femoral bones of a newly killed rabbit were broken, and the total marrow and blood mixture (2ml) was extracted with a syringe and diluted with an equal volume of DMEM (Dulbecco's Modified Eagle's Medium; Gibco, USA) containing 100 IU/ml penicillin, 100mg/ml streptomycin, 10% rabbit serum and 2mg/ml L-glutamine. The rabbit serum was prepared as follows: blood was collected into plastic tubes and kept at 4ºC for 30 min. The supernatant was centrifuged (8000 RPM for 10 min) for the extraction of clear serum. A micro-chip with its accessories (plastic tubes and bottles) was autoclaved at 90ºC for 30 min and then the temperature was reduced to 37(±0.1)ºC before the introduction of marrow cells by injection under pressure into the inlet of the microchip . Most of the cells (~100) were retained in the middle of the chamber. All culture media within the chip were sterilized by passage through 0.22 μm pore size filters inserted before the entrance to the microchip.

2.5 Culture of Alexandrium tamarense swimming cells

Single swimming cells of the marine toxic red tide dinoflagellate *A. tamarense* were incubated in culture medium (f/2) at a constant temperature of 20(±1)ºC. The injection cell density was 1.2×10^3/ml.

3 Results

3.1 Observation of flow.

Flow of a liquid in a chamber is invisible, even under a microscope. However, when the liquid is labeled by red cells, movement can be seen directly and is easy to record as time series images. Fig.S2A shows red cells in the flow near the entrance of the chamber. During a given length of time (1s or 3s), the distance traveled by each cell is different. There are two reasons to believe that red cells are slower than the maximal center velocity: flow is slower near the wall of the chamber than in the center; and cells touching the floor of the chamber under the influence of gravity are subject to forces of friction or adherence. For these two reasons, a single cell is incapable of labeling a flow. Fig.S2A shows the effect of pressure on the flow-rate at the entrance of the chamber. Fig.S2B shows the flow-rate of the red cells slowing during passage through the speed-control chamber.

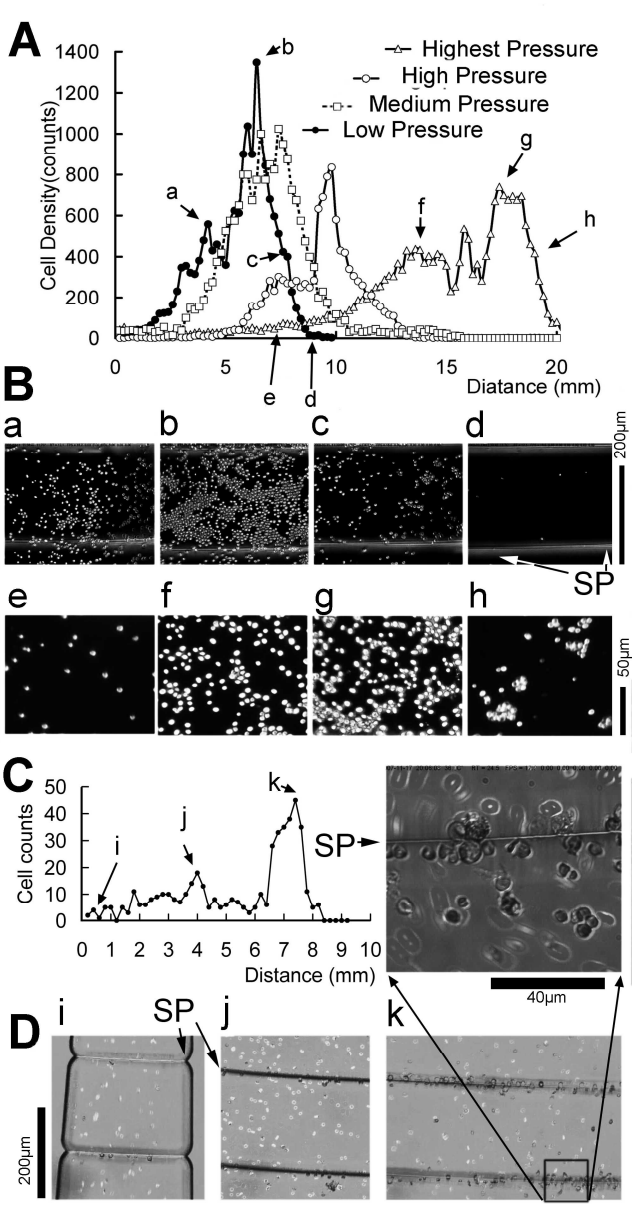

Fig.S3 The density distributions of different cells injected and controlled by pressure. **A** The peak of cell density (baker's yeast) moves from the entrance to the outlet in the speed-control chamber when the driven pressure is increasing. The images (25x phase contrast) at a, b, c, d, e, f, g, h along the curves of low pressure and highest pressure are shown in **B**. **C** The distribution of rabbit whole bone marrow cells in the speed-control chamber. Images at i, j, k on the curve are displayed in **D**. Two images 0.1s in succession are subtracted (by Adobe Photoshop CS) from each other to get a resulting image which shows the movements of any cell. Moving cells are white and cells at rest are black in **D**. The white cells (moving) are rabbit red cells and the black cells (at rest) are rabbit bone marrow cells. Red cells are not counted in **C**. SP：speed bumps.

Under constant pressure, the flow-rate at the entrance of the chamber is ~70 μm/s, which slows to ~20 μm/s, and eventually to about ~3μm/s. Some cells cannot pass over the speed bumps and are trapped in front of them. As the chamber has only one entrance

and one exit, the flow of uncompressible liquid never stops, even when it is too slow to push red cells over the speed bumps. The exceptional control of flow-rate in the chamber effectively retains even the most suspensible red cells in a constant flow, which suggests that no type of cell will be difficult to retain. Reagents and nutrients can be delivered continuously to any type of cell held in front of speed bumps, even when all cell types are present in the chamber simultaneously (even in fast flow) (for details see also PengSI_1.avi).

3.2 Microfluidic tests

The least-squares fit of the data (Fig.2A) is almost, but not quite, linear. Flow-rates increase more slowly below 200μm/s than above 200μm/s. As discussed above, red cells sinking to touch the floor of the chamber are subjected to forces of friction or adherence. When the flow-rate in the chamber increases over a series of bumps, streamlines along micro-chambers still follow the shape of the chamber. If the flow-rates are not very high (e.g. <200μm/s), gravity pulls cells to the floor and reduces their velocity. As the flow-rate increases (e.g. >200μm/s), inertia tends to keep moving cells flowing in a straight line, rather than following the zigzag streamlines along peaks and level floors. This inertia of cells works to withstand the force of gravity, and the effect of friction or adherence becomes weaker when the flow-rate increases, driven by increasingly higher pressure. The flow-rates of cells are not quite the same as those of the flow.

We used a high cell density (>10^4 cells /μl in distilled water) to find the peaks of cell distributions (Fig.3A and Fig.S3A). Each sample section was used for cell distribution analysis only once. The microchip was washed between sample section injections. We did not use only one sample section for further, deeper positions. Cells stop for several minutes of image taking, resulting in increasing adherence and those cells attached to the floor of the chamber do not fully re-suspend when they are pushed again for deeper entry. New injections of the sample present more valuable data.

The immotility of yeast cells does not cause the expansion of the peak. Liquids of different layers, according to the distance to the wall, have their own characteristic velocity; additionally, speed bumps disrupt the flow. The differences in velocity plus the differences in cell suspensibility and adherence are important in the process of peak expansion.

The peak of the left-hand sub-group is low (13.5mm), and the peak of the right-hand sub-group is high (17mm) at the highest pressure used. These four distributions are separate, single-sample injections; they are not the same cells and have no causal relationship. So, these independent distributions provide strong evidence of two sub-groups of yeast cells.

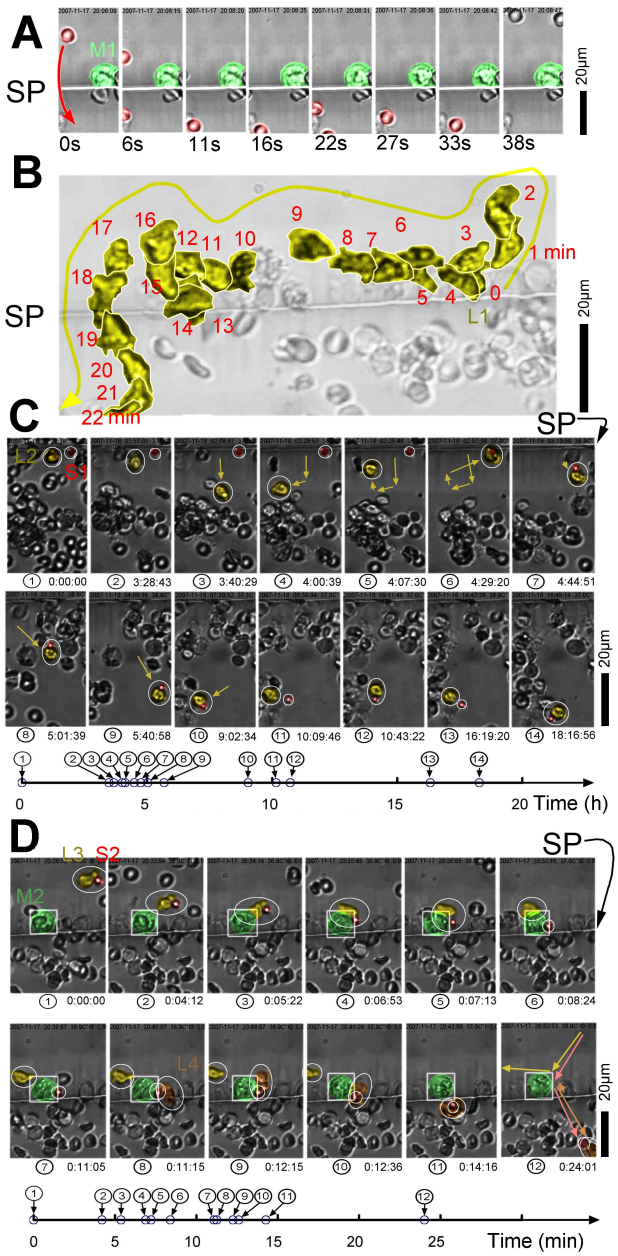

Fig.S4 Rabbit bone marrow cells in a speed-control chamber. Images are extracted from video clips (see PengSI_02.avi, PengSI_03.avi, PengSI_04.avi and PengSI_05.avi). Flows in all pictures are from North to South. Cells discussed in the text are manually coloured in yellow or orange (leukocyte), green (big bone marrow cell) and red (big red cells in **A** or small bone marrow cell in **C** and **D**). **A** The spinning of a bone marrow cell (green, M1) driven by a micro flow. The flow labelled by an arc arrow starts at 0s and two moving red cells (red) show the increasing micro flow. **B** The path of a moving leukocyte (yellow, L1). Numbers along the path are a time sequence in minutes. **C** A long-term observation of a leukocyte's differentiation, chemotaxis, catching and carrying processes (yellow L2 in a big white circle and red S1 in a small white circle). Circulated numbers and time below each image are also depicted on a time axis to show the relative time of each image event (similarly hereinafter). **D** A leukocyte (yellow, L3) carries and delivers a small bone marrow cell (red, S2) to a big bone marrow cell (green, M2), which is successively snatched by another leukocyte (orange, L4). Arrows show the paths of moving cells. **SP**: speed bumps.

3.3 Multi-cell culture of rabbit bone marrow aspirate

Flows in mammalian bodies are permanent, like the blood in blood vessels, a perpetual motion that maintains life. With precise velocity control, liquid around cells is adjusted back and forth to see if the cell's spinning is caused by the flow. A large number of rabbit bone marrow cells were observed spinning in flows. When the flow was stopped, the cells stopped spinning. Fig.S4A shows the control of a start of flow around a cell (M1) and the cell started to spin again 16s later (see also PengSI_2.avi).

Amoeba-like white blood cells (leukocytes) have a high degree of motility and pass freely through the narrow gaps among vascular cells. The gap over a speed bump is very similar to the gaps among vascular cells; it prevents the passage of bone marrow cells but is never too narrow for leukocytes. Fig.S4B shows the route of a leukocyte (L1) after 20 min, while this leukocyte moved freely at speeds of about 10μm/min, other bone marrow cells did not move (see also PengSI_3.avi). The cross-bump movement showed the high degree of motility of this type of cell, which still cannot escape from the inlet because the flow near the entrance is strong.

Fig.S4C and D are details of Fig.4A and B in the paper.

3.4 Swimming cell cultures of dinoflagellate cells

Fig.S5 is detail for fig.4D. A temporary cyst is an asexual life-cycle stage common in dinoflagellates. The formation of temporary cysts is frequently associated with stress. Germination occurred from cysts within 24 h if a new medium and ample food were provided. Germination of temporary cysts usually occurred from 30 min to 5 h after the cysts were formed. Fig.S5 shows 9 cysts incubated in front of a speed bump. Video clips show that 7 of these 9 cysts (> 70%) germinate. All of these 7 newly germinated cells have new circumvolving flagellum. They squeeze over the speed bump and go downstream. These seven newly germinated cells have a swimming speed of about 50–100μm/s (~4–8m/d), similar to its sinking speed (8.6 m/d). The fast flow suppresses the motility of cells and excysted cells; no matter what direction they germinate toward, they finally turn downstream and go deeper inside the chamber (see the white routes with black arrowheads in Fig.S5). Each of these 7 excysted cells has its own germination sequence image in 30s intervals (see Fig 6E). The germination times are labeled in Fig.S5B (see also PengSI_6.avi). Germination processes are too fast to be observed by the very long video recording time (over 24 h or even several days). The duration of excystment was never longer than 150s (90~150s). The range of the 7 germination times was 7 – 16 h. The video analysis shows a very high germination rate of > 70%. (See PengSI_6.avi).

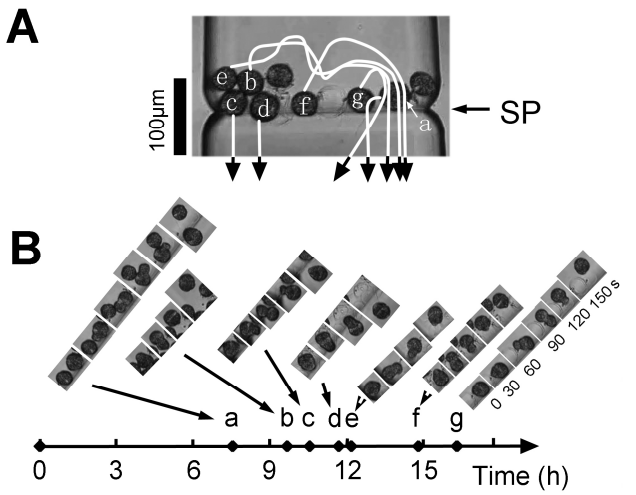

Fig.S5 Cultured fast swimming single cells (*Alexandrium tamarens*) in a speed-control chamber. **A** The paths of single cells germinating from their cysts and going across the speed bumper. Every cell is labelled by a, b, c, d, e, f and g for references. **B** Time-lapse germination sequences of 7 cysts (from left to right, 30s interval). **SP**： speed bumps.