Supplementary information

A microfluidic design to provide stable and uniform in-vitro microenvironment for cell culture inspired by the redundancy characteristic of leaf areole

Jingmin Li,^{‡a} Juan Wei,^{‡a} Yuanchang Liu,^b Bo Liu,^c Tao Liu,^a Yang Jiang,^a Laiqian Ding^a and Chong Liu^{*ad}

^a Key Laboratory for Micro/Nano Technology and System of Liaoning Province, Dalian University of Technology, Dalian, 116023, P. R. China.

^b Department of Mechanical Engineering, University College London, London, UK, NW12BX

^c Department of Biomedical Engineering, Dalian University of Technology, Dalian, 116023, P. R. China

^d Key Laboratory for Precision and Non-traditional Machining Technology of Ministry of Education, Dalian University of Technology, Dalian, China

‡These authors contributed equally to this work

*Correspondence and requests for materials should be addressed to Chong Liu (Email: chongl@dlut.edu.cn)

Supplementary information S1: The numbers of different polygonal areoles in six kinds of plants.



Supplementary Figure 1. The numbers of different polygonal areoles in six kinds of plants. (a) The sampling regions on an alamo leaf. Six regions (labeled with 1, 2, 3, 4, 5, 6) are selected. Region 1, region 2, region 3 are in the symmetrical location with region 6, region 5, region 4. The locations of region 1, region 2 and region 3 are (L/4, $W_{L/4}/2$), (L/2, $W_{L/2}/2$), (3L/4, $W_{3L/4}/2$), respectively. Where L is the length of a leaf, W is width. The sampling regions of other plants are selected using the same method. (b) The leaf venation regions sampled from 1 to 6. The pictures are collected by a microscope (Olympus STM6, Japan). The scale bar is 500 µm. (c-h) The ratios of the number of different polygonal areoles to the total number of areoles in the selected region. It is maple, peach, chinar, Alamo, sakura and Chinese redbud successively from (c) to (h).

Supplementary information S2: The octagonal chambers with different number of microgaps.

By mimicking the pits on vessels, the microgaps are designed to connect the channels with chambers. To study the effects of the number of microgaps, eight types of microfluidic devices with different number (2, 4, 8, 16, 24, 32, 40, 48) of microgaps are fabricated. These eight types of devices have the same geometry sizes except the number of microgaps. The inscribed circle diameters of these octagonal chambers are all 1 mm, and the depths are 60 μ m. The depths and widths of main channels, bifurcated channels and microgaps are 60 μ m*200 μ m, 60 μ m*69 μ m and 60 μ m*6 μ m, respectively.



Supplementary Figure 2. The octagonal chamber with different number of microgaps. (a) Chamber with two microgaps. (b) Chamber with four microgaps. (c) Chamber with four microgaps. (d) Chamber with sixteen microgaps. (e) Chamber with twenty-four microgaps. (f) Chamber with thirty-two microgaps. (g) Chamber with forty microgaps. (h) Chamber with forty-eight microgaps. The scale bar is 300 µm.

Supplementary information S3: The octagonal chambers with different size of microgaps.

To investigate the influence of the size of microgaps, four types of chambers are designed. The sizes of microgaps are 100 μ m*5 μ m*60 μ m, 100 μ m*6 μ m*60 μ m, 100 μ m*7 μ m*60 μ m, 100 μ m*9 μ m*60 μ m, 100 μ m*11 μ m*60 μ m, respectively. The other sizes are the same with Supplementary Fig. 2f.



Supplementary Figure 3. The octagonal chamber with different size of microgap. (a) Chamber with circumferential width of the microgap being 5 μ m. (b) Chamber with circumferential width of the microgap being 7 μ m. (c) Chamber with circumferential width of the microgap being 9 μ m. (d) Chamber with circumferential width of the microgap being 11 μ m. The scale bar is 300 μ m.

Supplementary information S4: The octagonal chambers with different inscribed circle diameter.

The area of cell culture chamber will affect the flow field and the efficiency of changing the medium. We should find an optimized area to balance the uniformity and stability of flow field with the changing efficiency. Four types of chambers with diffident inscribed circle diameters (500 μ m, 1000 μ m, 1500 μ m and 2000 μ m) are designed. The sizes are the same with Supplementary Fig. 2f except the inscribed circle diameters of chambers.



Supplementary Figure 4. The octagonal chamber with different size of inscribed circle diameter. (a) Chamber with 500 μ m inscribed circle diameter. (b) Chamber with 1000 μ m inscribed circle diameter. (c) Chamber with 1500 μ m inscribed circle diameter. (d) Chamber with 2000 μ m inscribed circle diameter. The scale bar is 300 μ m.

Supplementary information S5: The trajectories of particles in the polygonal chambers with different number of edges.

The movements of particles in the square chamber, pentagonal chamber, hexagonal chamber, heptagonal chamber, nonagonal chamber and decagonal chamber are recorded by the CCD camera with an interval of 50ms. The pictures are dealt with Image Pro Plus 6.0 to draw the trajectories of PS microbeads. The duration in different chambers are 20 s, 20 s, 20 s, 10 s, 10 s, 10 s.



Supplementary Figure 5. The particle trajectories in chambers with different shape. (a) Square. (b) Pentagon. (c) Hexagon. (d) Heptagon. (e) Nonagon. (g) Decagon. The scale bar is 200 µm.

Supplementary information S6: The trajectories of particles in the octagonal chambers with different number of microgaps.

The movements of particles in the octagonal chambers with different number of microgaps (2, 4, 8, 16, 24, 32, 40, 48) are recorded by the CCD camera with an interval of 50ms. The pictures are dealt with Image Pro Plus 6.0 to draw the trajectories of PS microbeads. The duration in different chambers are 140 s, 80 s, 40 s, 30 s, 20 s, 20 s, 15 s, 15 s.



Supplementary Figure 6. The particle trajectories in chambers with different number of microgaps. (a) Two. (b) Four. (c) Eight. (d) Sixteen. (e) Twenty-four. (f) Thirty-two. (g) Forty. (h) Forty-eight. The scale bar is 100 µm.

Supplementary information S7: The trajectories of particles in the octagonal chambers with different size of microgaps.

The movements of particles in the octagonal chambers with different size of microgaps (5 μ m, 7 μ m, 9 μ m and 11 μ m) are recorded by the CCD camera with an interval of 50ms. The pictures are dealt with Image Pro Plus 6.0 to draw the trajectories of PS microbeads. The duration in different chambers are 20 s, 10 s, 3 s, 2 s.



Supplementary Figure 7. The particle trajectories in chambers with different size of microgaps. (a) 5 µm. (b) 7 µm. (c) 9 µm. (d) 11 µm. The scale bar is 200 µm.

Supplementary information S8: The trajectories of particles in the polygonal chambers with different inscribed circle diameter.

The movements of particles in the octagonal chambers with different inscribed circle diameter (500 μ m, 1000 μ m, 1500 μ m and 2000 μ m) are recorded by the CCD camera with an interval of 50ms. The pictures are dealt with Image Pro Plus 6.0 to draw the trajectories of PS microbeads. The duration in different chambers are 10 s, 20 s, 30 s, 40 s.



Supplementary Figure 8. The particle trajectories in chambers with different inscribed circle diameter. (a) 500 μ m. (b) 1000 μ m. (c) 1500 μ m. (d) 2000 μ m.

Supplementary information S9: The fluid field comparison of octagonal chamber without redundancy and the bio-inspired octagonal chamber.

The pits on the side wall of vessels have significance on improving the redundancy of the water transportation in the leaf venation network. In order to investigate the effects of pits on the fluid field in areole, the octagonal chamber without redundancy is designed to compare with the bio-inspired octagonal chamber.



Supplementary Figure 9. The fluid field comparison of octagonal chamber without redundancy and the bio-inspired octagonal chamber. (a) The structure of the octagonal chamber without redundancy. The only difference with the bio-inspired octagonal chamber (Fig 3a) is the gap. There are two gaps distributed uniformly on the wall of the chamber, and the width of the gaps are 100 μ m. As a comparison, there are thirty-two gaps distributed uniformly on the wall of the bio-inspired octagonal chamber, and the width of the gaps are 6.25 μ m. (b) The time dependent simulation results of the average velocity in the octagonal chamber without redundancy and the bio-inspired octagonal chamber. The inlet velocity is $V_{inlet}=6950+2780*sin(\pi^*t)$ μ m/s. The results show that the average velocity in the octagonal chamber. It indicates that the fluid field in the bio-inspired octagonal chamber is stable than octagonal chamber without redundancy. (c) The stationary simulation results of velocity field in the octagonal chamber without redundancy. The inlet velocity is 6950 μ m/s. Line A-A' is the link of the midpoints of the two gaps and line B-B' is the perpendicular line of A-A'. The velocity along line A-A' and B-B' are calculated. The velocity field in the bio-inspired octagonal chamber without redundancy is not uniform. (d) Steady-state simulation results of velocity in the octagonal chamber without redundancy is not uniform. (d) Steady-state simulation results of velocity in the octagonal chamber without redundancy is not uniform. (d) Steady-state simulation results of velocity in the inlet velocity is 6950 μ m/s. Line C-C' is the link of the midpoints of the two symmetrical gaps and line D-D' is the perpendicular line of C-C'. The velocity along line C-C' and D-D' indicate that nearly 90% of the velocity in the bio-inspired octagonal chamber.

Supplementary information S10: The Hela cells cultured in BOD and COD with different inlet flow rates.

In order to investigate the influence of flow field on cell proliferation and attachment, the average velocity in the control octagonal chamber has been reduced to the magnitude of bio-inspired octagonal chamber. The Hela cells are cultured in bio-inspired octagonal device with inlet flow rate of 5 ul/min (BOD), control octagonal device with inlet flow rate of 5 ul/min (COD #1) and control octagonal device with inlet flow rate of 0.28 ul/min (COD #2) respectively. The states of cells in different chambers are recorded. The normalized cell density and average attachment area of a single cell in BOD, COD #1 and COD #2 are measured after culturing for 54h.



Supplementary Figure 10. The effects of flow rate on Hela cell density and average attachment area of a single cell in different devices. (a) The Hela cells are cultured in the bio-inspired octagonal device (BOD). The medium inlet flow rate is 5μ l/min. (b) The Hela cells are cultured in the control octagonal device (COD). The medium inlet flow rate is 5μ l/min. (c) The Hela cells are cultured in the control octagonal device (COD). The medium inlet flow rate is 5μ l/min. (c) The Hela cells are cultured in the control octagonal device (COD). The medium inlet flow rate is 5μ l/min. (c) The Hela cells in BOD, COD #1 and COD #2 are measured after culturing for 54h. The ** and # indicate statistical significance (P<0.01), (n=5) and (P<0.05), (n=5) respectively. (e) The average attachment area of a single cell in BOD, COD #1 and COD #2 are measured after culturing for 54h. The ** and # indicate statistical significance (P<0.05), (n=4).

Supplementary information S11: Alignment of PC-12 cells.

Most of in-vivo cells live in interstitial flow. The creeping fluid flow has been shown to affect the morphology of cells. The results of particle tracking experiments in the study indicate that the bio-inspired device can control the flow field inside the culture chamber comparable to interstitial flow. In order to investigate the influences of the interstitial-like flow on cell alignment, PC-12 cells are cultured in the bio-inspired octagonal device (BOD). The culture dish (CD) and control octagonal device (COD) are used as comparisons. The cells are cultured in static for 6h after seeding into the culture devices, then the PC-12 cells in BOD and COD are cultured dynamically with the medium inlet flow rate of 5µl/min for 36h. The state of cells are recorded after culturing for 18h and 42h.



Supplementary Figure 11. Alignment of PC-12 cells. (a) Steady-state simulation results of velocity field in the bio-inspired octagonal device (BOD). The inlet velocity is 6950µm/s (5µl/min). The red arrows are the flow directions in the main channel. The black lines are the streamlines. (b) Steady-state simulation results of velocity field in the control octagonal device (COD). The simulation conditions are same with Supplementary Fig. 11a. (c) The PC-12 cells are cultured in the culture dish (CD) statically for 42h. (d) The PC-12 cells are cultured in the bio-inspired octagonal device (BOD) for 42h. The cells are cultured in static for 6h after seeding into the culture chamber, then cultured dynamically with the medium inlet flow rate of 5µl/min for 36h. The state of cells are recorded after culturing for 18h and 42h. The red arrow is the flow direction in the main channel. (e) The PC-12 cells are cultured in the control octagonal device (COD) with the same condition of Supplementary Fig. 11d.