Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information

Live cell imaging analysis of the epigenetic regulation of the human endothelial cell migration at single cell resolution

Chunhong Zheng,[a,b,1] Zhilong Yu,[a,b,1] Ying Zhou,[a,b,1] Louis Tao,[c] Yuhong Pang,[a,b] Tao Chen,[a,b] Xiannian Zhang,[a,b] Haiwei Qiu,[a,2] Hongwei Zhou,[a,b] Zitian Chen,[b,d] and Yanyi Huang[a,b,d,2]

[a] College of Engineering, Peking University, Beijing 100871, China.
[b] Biodynamic Optical Imaging Center (BIOPIC), Peking University, Beijing 100871, China.
[c] Center for Bioinformatics, National Laboratory of Protein Engineering and Plant Genetics Engineering, College of Life Sciences, Peking University, Beijing 100871, China.
[d] College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China.

[1] These authors contributed equally to this work.
[2] To whom correspondence should be addressed. E-mail: yanyi@pku.edu.cn.

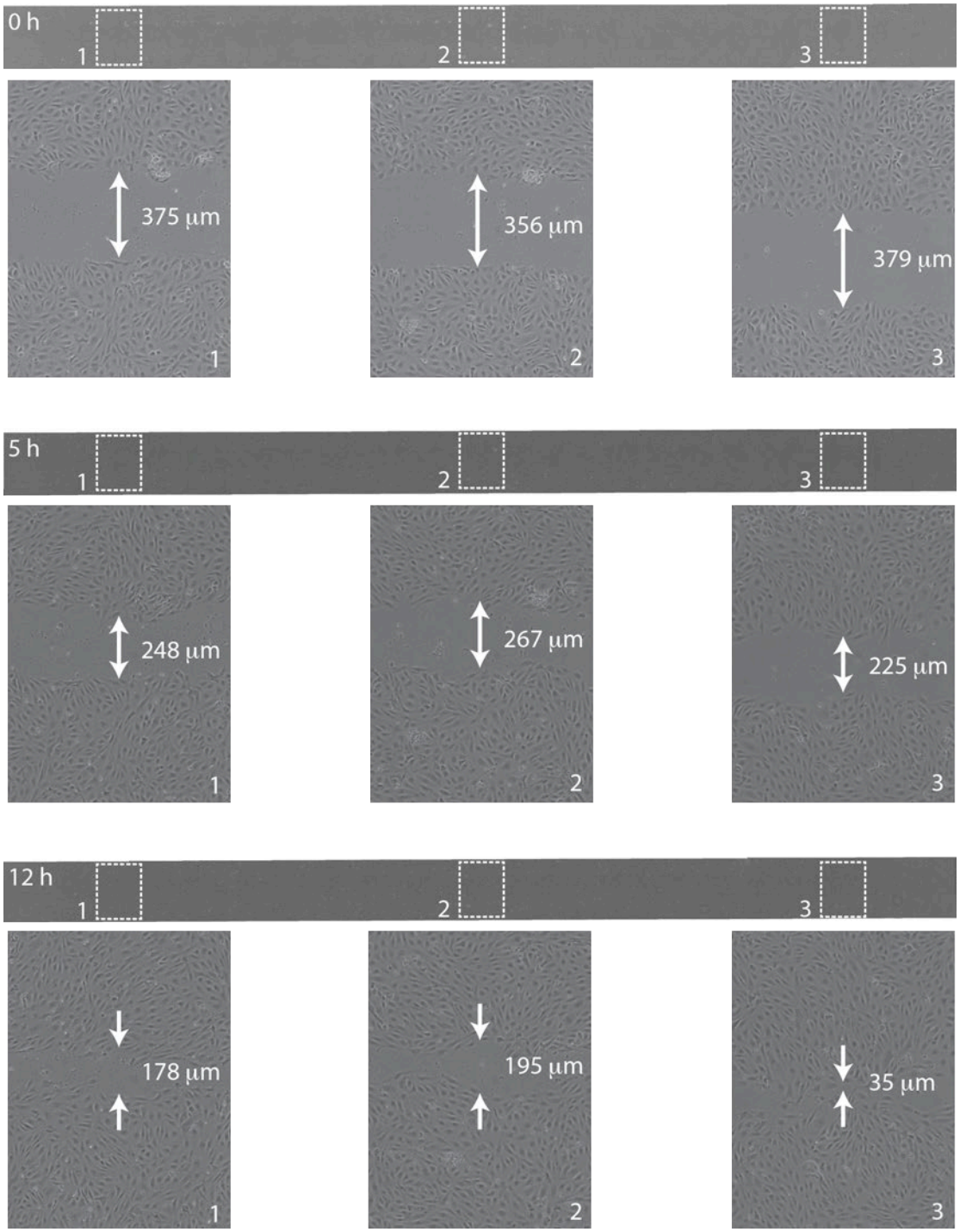


Fig. S1. We show a typical scratch on a layer of HUVECs and the "healing" process with time, using the conventional scratch assay. In the experiment we need to pick 50 spots along the scratch and then measure the remaining width of the gap. We show zoomed pictures of three spots at 0 h, 5 h, and 12 h after the scratch generation, demonstrating the dispersive distribution of the gap width along cell migration.

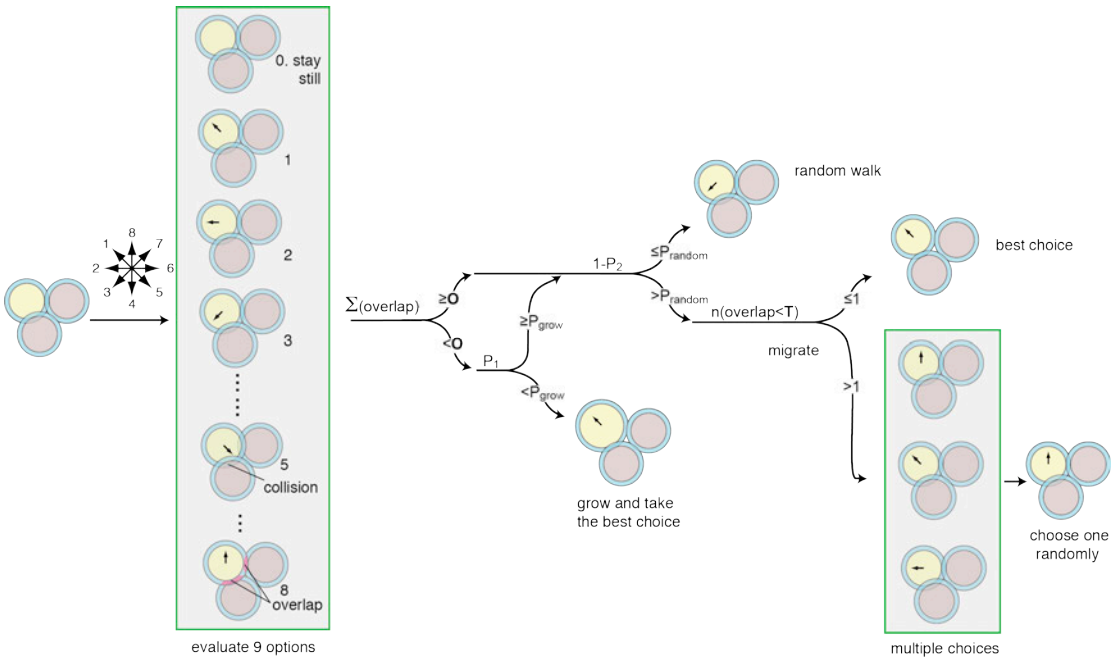


Fig. S2. A numerical method has been developed to simulate the cell motion. We evaluate 9 different moving options for each individual cell at each step, and then choose one option to avoid collision. For each direction, the cells will have certain possibility to grow when migrating, as we have observed in the experiments.

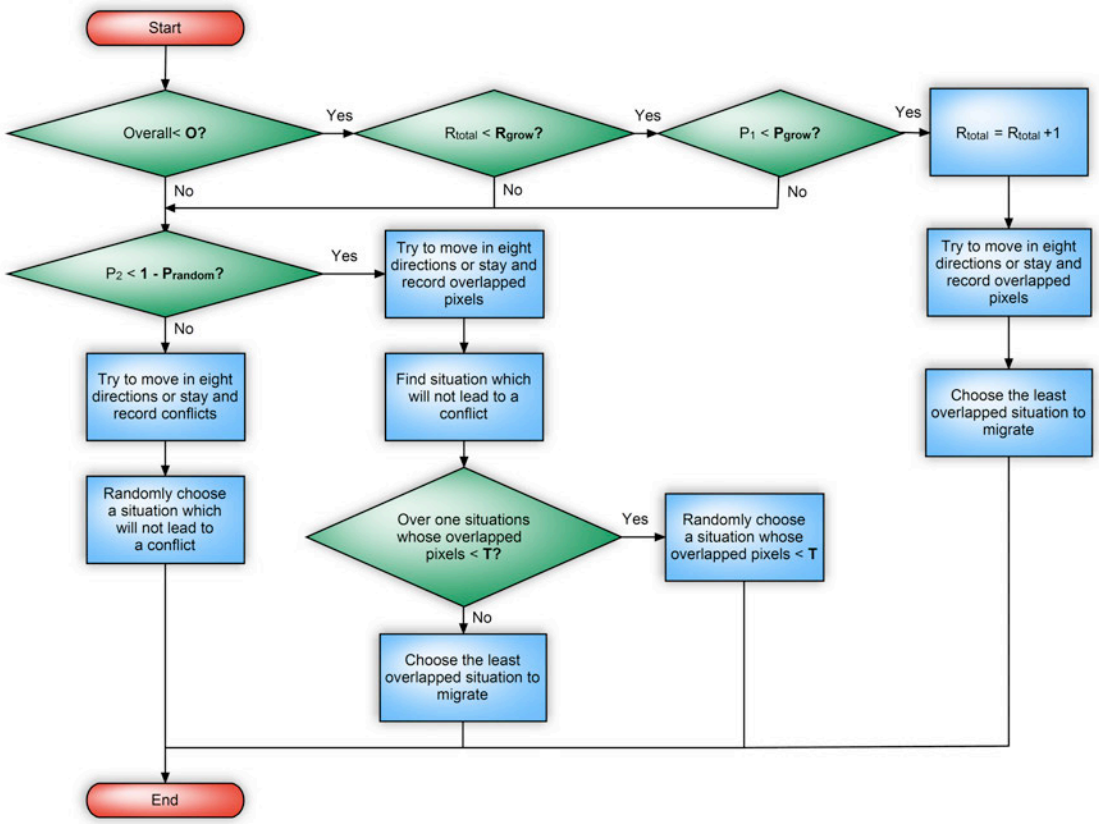


Fig. S3. The flow chart of numerical simulation. The detailed description is in the Supplementary Methods.

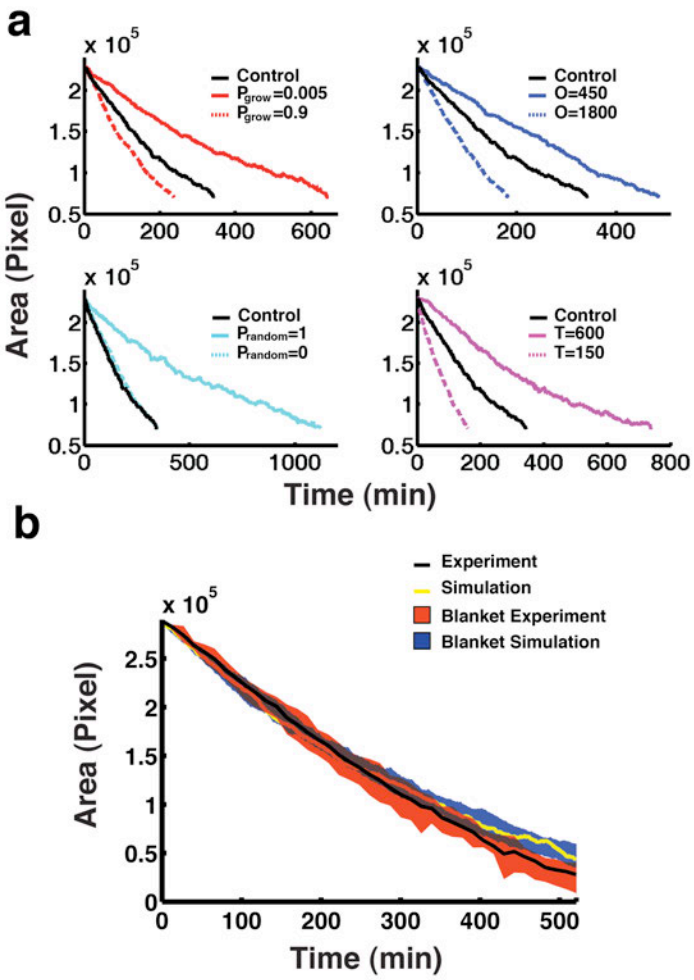


Fig. S4. Simulation of blank-filling through the particle model. (A) The effect of different parameters for simulation. All black lines are generated from simulations with the same set of parameters (P_{grow} = 0.8, P_{random} = 0.2, O = 900, T = 300), labeled as “control” group. Color lines (solid and dashed) represent simulation results with different parameters. For each subgroup, one parameter is changed while keeping the other three the same of the control group. (B) The stochastic effect of numerical simulation is demonstrated by fifty independent runs using same parameters. The blue blanket represents the result of fifty simulation runs and the red blanket shows the experimental result.

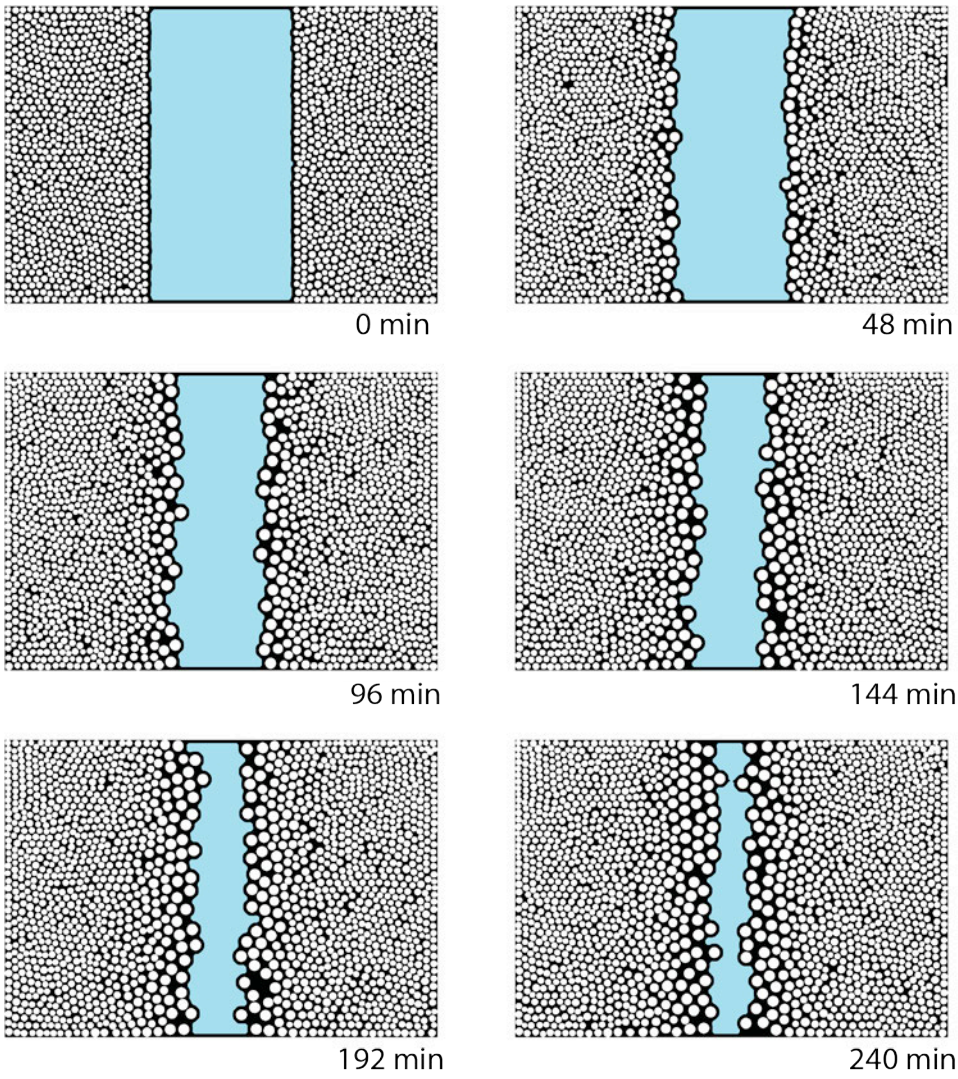
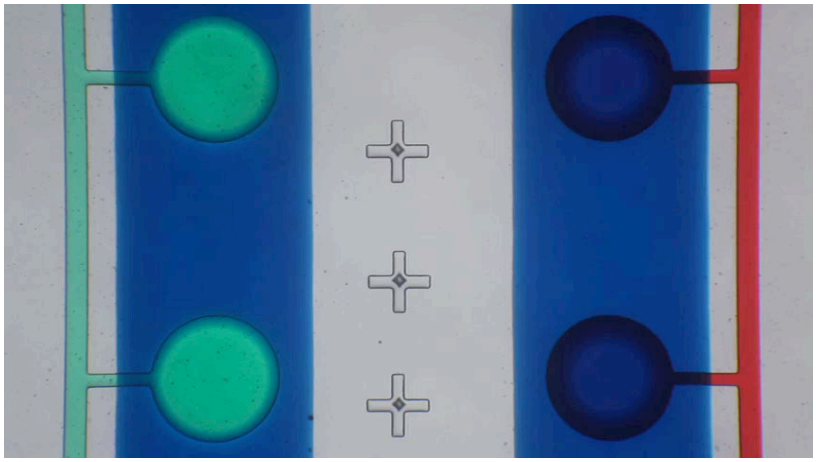


Fig. S5. Simulation of the scratch assay (550 μm gap) using particle model, showing that the wound gap (cyan) becomes irregular during the migration.

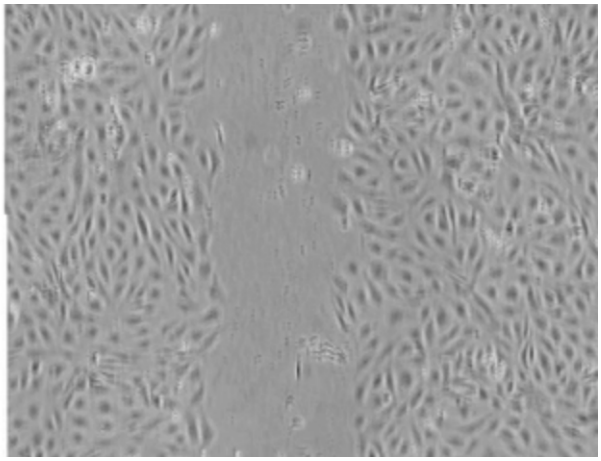
Code #1 (

SI Movie 1.



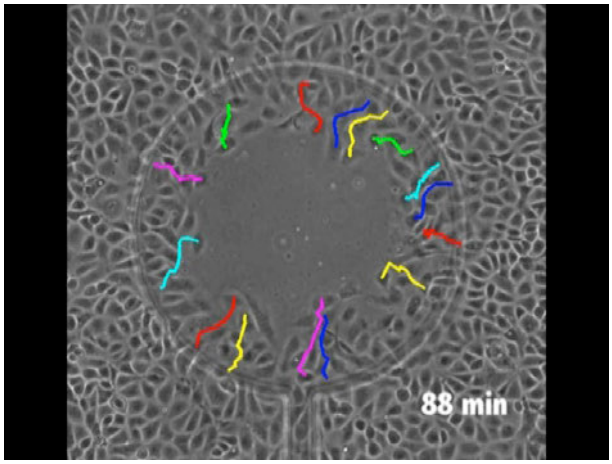
The button valves are actuated by pneumatic pressure. This movie shows two individually controlled groups of button valves driven sequentially. The buttons are filled with red or green dyes while the culture channels are filled with blue dye. During the actuation, the liquid inside the culture channel but beneath the buttons will be pushed away; hence the buttons will turn into red or green.

SI Movie 2.



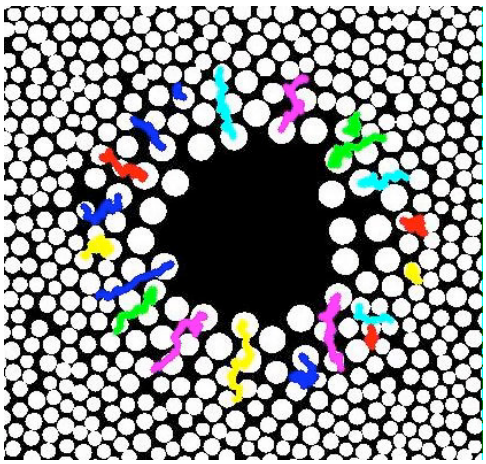
The microscopic view of a wound generated by scratch assay at different time points during the experiments. With cell migration, the irregularity of the wound edge is creased, causing difficulty to measure the gap width.

SI Movie 3.



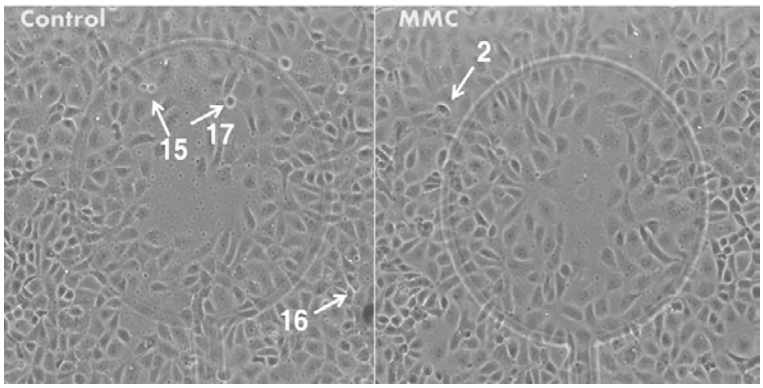
The blank-filling process around a button. The video is generated from a series of phase contrast microscopic images. The positions of a few cells around the edge of the blank are identified in each image and the trajectories of these cells are marked in the video.

SI Movie 4.



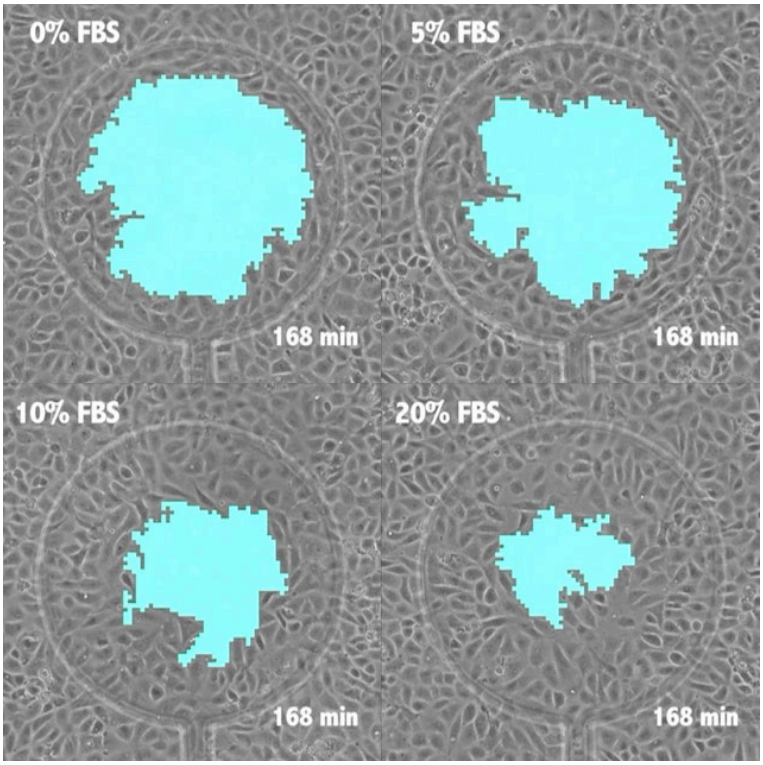
The simulated blank-filling process. During the simulation, each cell will choose its moving direction, or grow, based on a particle model described in the supplementary methods. The positions of a few cells around the edge of the blank are identified in each image and the trajectories of these cells are marked in the video.

SI Movie 5.



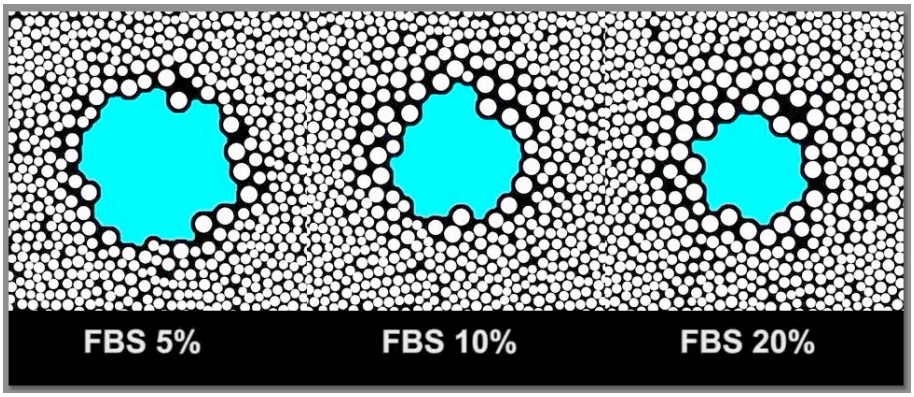
The blank-filling process of HUVECs with and without MMC treatment. Each cell-dividing event within the field of view has been identified and counted. MMC will inhibit cell proliferation, hence less cell dividing events are recorded. We use this method to quantitatively measure the proliferation rate of HUVECs on-chip and notice that with normal culture condition the proliferation will not make significant contribution to the blank-filling with a short experimental time.

SI Movie 6.



Time-lapse videos of HUVECs on a single chip with different FBS concentration show that the cell motility is affected by the serum concentration.

SI Movie 7.



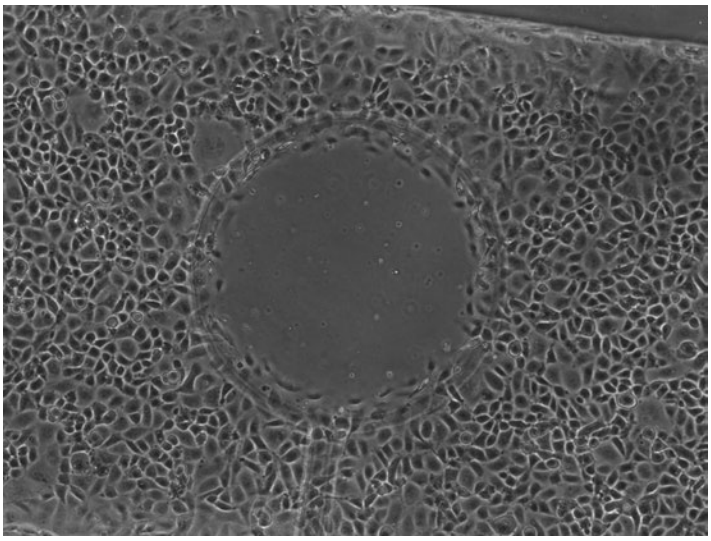
The simulated time-lapse images of cell migration with different motility. These videos are used to fit the experimental data for calculate the cell migration speed of single cells.

Supporting codes for image processing (Matlab script)

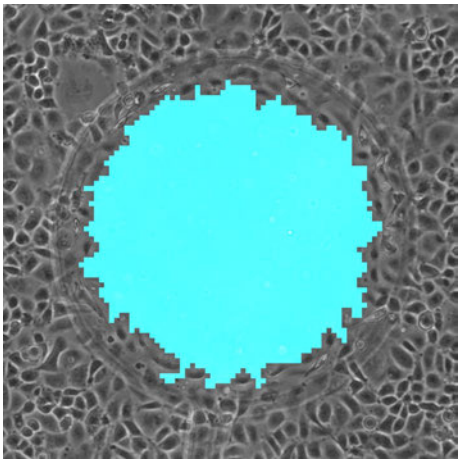
```
% the code of "calcuArea .m"
% calcuArea.m calculate the areas of blank units in NUM time-lapse images.
% A median filter is applied to the picture N times.
% THRESH is the threshold for the variance of the intensity to differentiate cell
unit from blank unit.
% SIZE×SIZE pixel sections are cropped from the original time-lapse images for
image processing.
clc;
clear;
%%
N=2; THRESH=16; NUM=5; SIZE=900;
inPic=imread([int2str(1),'.jpg']);          % load the first image of each picture
series.
for k=1:N
   inPic=medfilt2(inPic);              % apply an median filter to the picture N times
end
[Centroid,bwPic]=gray2bwPic(inPic, THRESH);   % transform a grayscale image
to a binary image
showPic(:,:,1)=inPic;                  % display the picture to adjust THRESH
showPic(:,:,2)=inPic+uint8(bwPic*255);
showPic(:,:,3)=inPic+uint8(bwPic*255);
imshow(showPic);
hold on;
plot(Centroid(1),Centroid(2),'*')
clear showPic;

%%
for picNum=1:NUM
  inPic=imread([int2str(picNum),'.jpg']);
     inPic=imcrop(inPic,[Centroid(1)-SIZE/2,Centroid(2)-SIZE/2,SIZE-1,SIZE-1]);
                              % crop SIZE*SIZE pixel sections from the original time-
lapse images
  [serialCentroid,bwPic]=gray2bwPic(inPic, THRESH);
  buttonArea(picNum)=sum(sum(bwPic));
  showPic(:,:,1)=inPic;
  showPic(:,:,2)=inPic+uint8(bwPic*255);
  showPic(:,:,3)=inPic+uint8(bwPic*255);
  imwrite(showPic,[int2str(picNum),'.processed.jpg']);
end
xlswrite(['buttonArea.xls'],[buttonArea]);

% the code of "gray2bwPic .m"
% gray2bwPic.m is a son function of calcuArea.m to transform a grayscale image
to a binary image and calculate the centroid of the blank region.
function [Centroid,bwPic]= gray2bwPic (inPic, threshold)
for i = 1:size(inPic,2)/10
```

```
  for j = 1:size(inPic,1)/10
    h_index = (i-1)*10+1;
    l_index = (j-1)*10+1;
    subPic = double(imcrop(inPic,[h_index,l_index,9,9]));  % divide an original
image into 10×10 pixel units
    tmp = reshape(subPic,10*10,1);
    Variance = var(tmp);          % calculate the intensity variance for each unit
    if Variance < threshold        % use the threshold to determine the unit
belongs to cell region or blank region
       inPic(l_index:l_index+9,h_index:h_index+9) = 1;
    else
                inPic(l_index:l_index+9,h_index:h_index+9) = 0;
    end
  end
end
bigArea = bwareaopen(inPic,6000,4);     % pick the biggest connected region and
fill all the speckles inside this region as the blank area
bigArea = imfill(bigArea,'holes');
bigArea = bwlabel(bigArea);
picStat = regionprops(bigArea,'Area','Centroid');
for k = 1:max(max(bigArea))
  conArea(k,1) = picStat(k,1).Area;
end
bigAreaIndex = find(conArea==max(conArea));
Centroid = picStat(bigAreaIndex,1).Centroid;
bwPic = bigArea == bigAreaIndex;
```

Note: The input should be an image series containing NUM images with the name '1.jpg', '2.jpg', …'NUM.jpg'. The output are the processed square images cut from the original images with blank regions labeled in cyan and a excel file with the corresponding blank region areas. For example, if the input is 1.jpg, the output will be 1.processed.jpg and buttonArea.xls containing the blank area '249600'.



1.jpg



1.process.jpg