Supporting Information

Microfluidic Covalent Immobilization of Multi-gradient RGD Peptides on a Gelatin Surface for Studying Endothelial Cell Migration

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1 Supplementary methods

Analysis of cell orientation along the gradient direction

After 24 h of HUVEC seeding on the gelatin surface with the RGD peptide concentration gradient, the cells were captured using a microscope equipped with a CCD camera. Subsequently, ImageJ software and Matlab was utilized to analyze the images and determine the angles between the major axis of cells and the direction of the RGD peptide concentration gradient (Fig. S11 and Fig. S10).[1] Three to five independent experiments were conducted, and approximately 100 cells were selected for cell orientation calculation in each experiment. The angles between the major axis of the cells and the gradient direction were categorized into nine groups, with each group covering a range of 10 degrees (0°-90°).

2. Supporting Figures

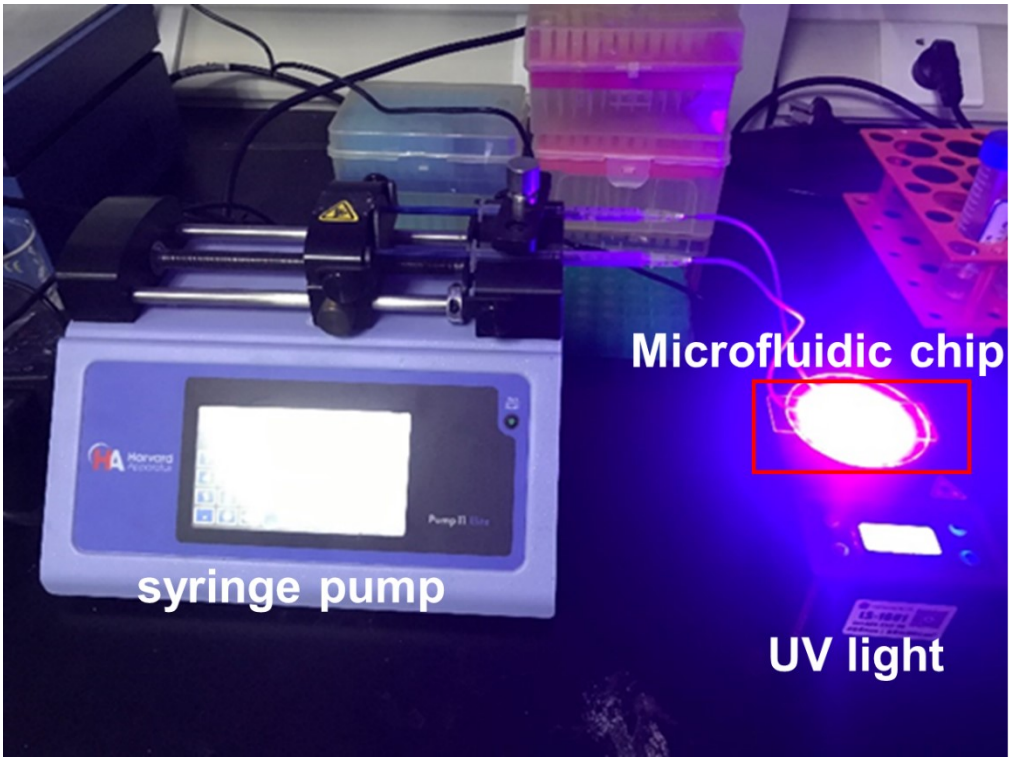

Fig. S1. Real picture of the experimental platform and real picture of the proposed microfluidic chip.

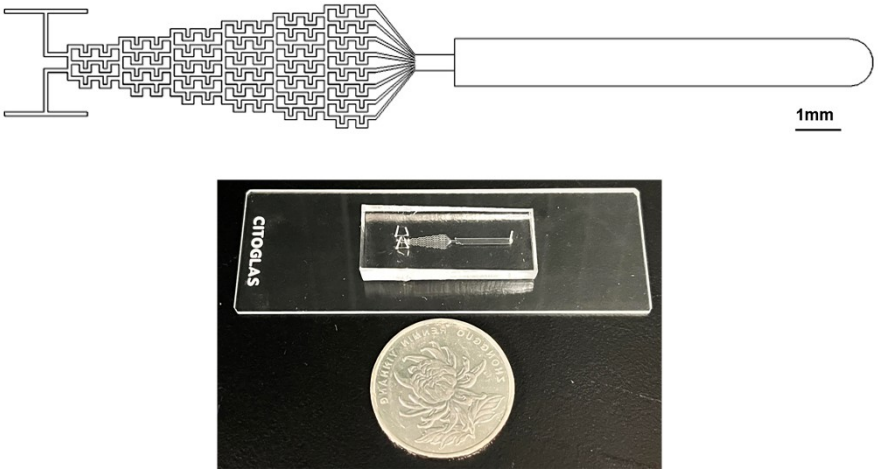

Fig. S2. Schematic representation of the microfluidic design and real picture of the proposed microfluidic chip.

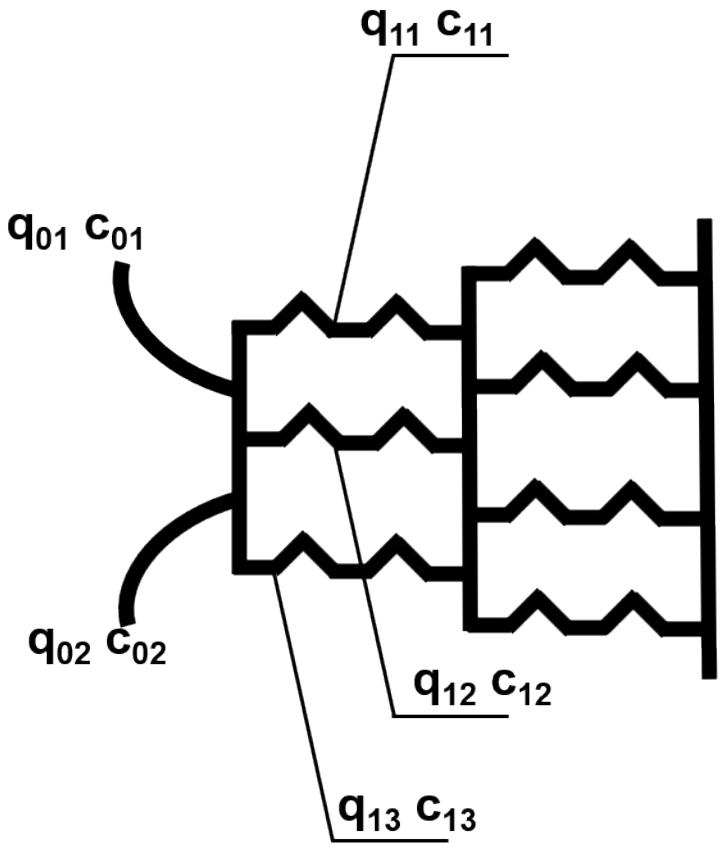

Fig. S3. Schematic illustration of a traditional "Christmas tree" structure for generating spatially monotonic concentration gradients.

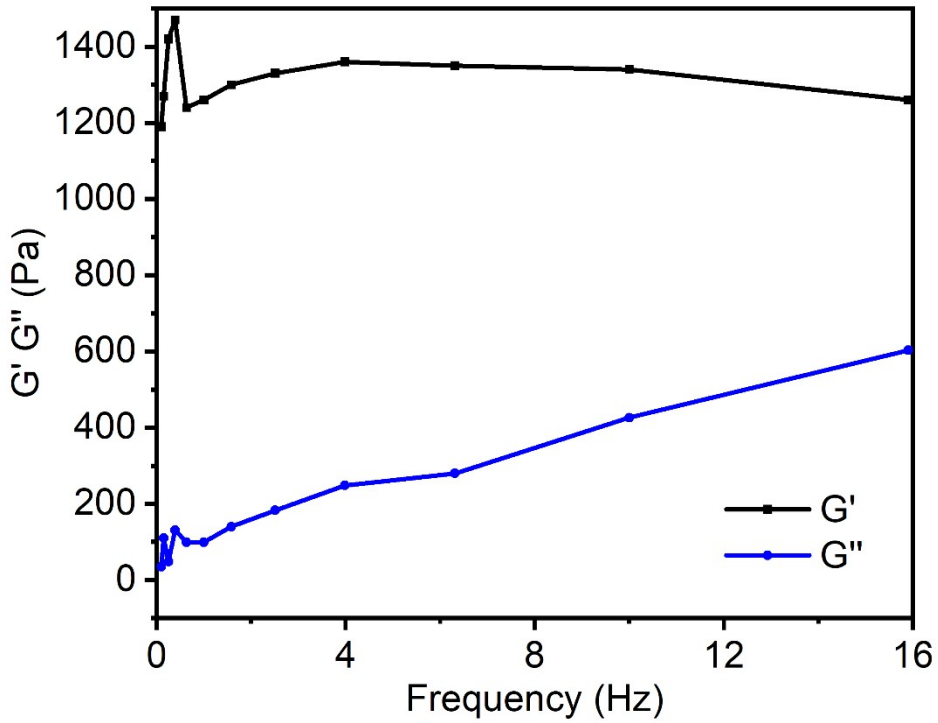

Fig. S4. Quantitative analysis of final G’ and G’’ of GelMA after photo-crosslinking.

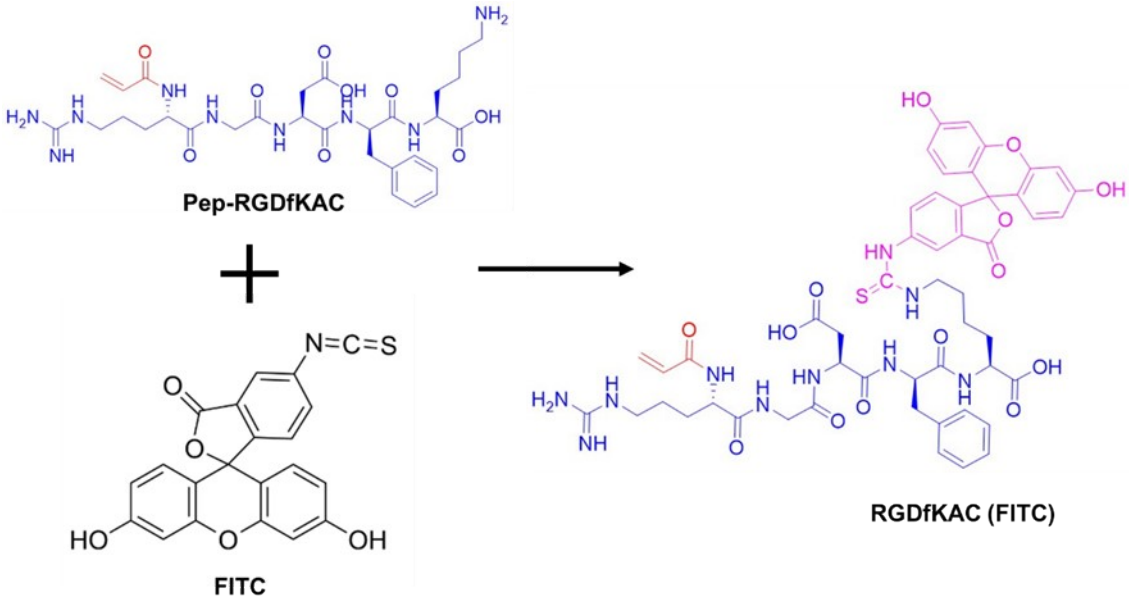

Fig. S5. The peptides RGDfKAC where conjugated with FITC biocytin to introduce a biotin-tag and the chemical structures of the conjugates.

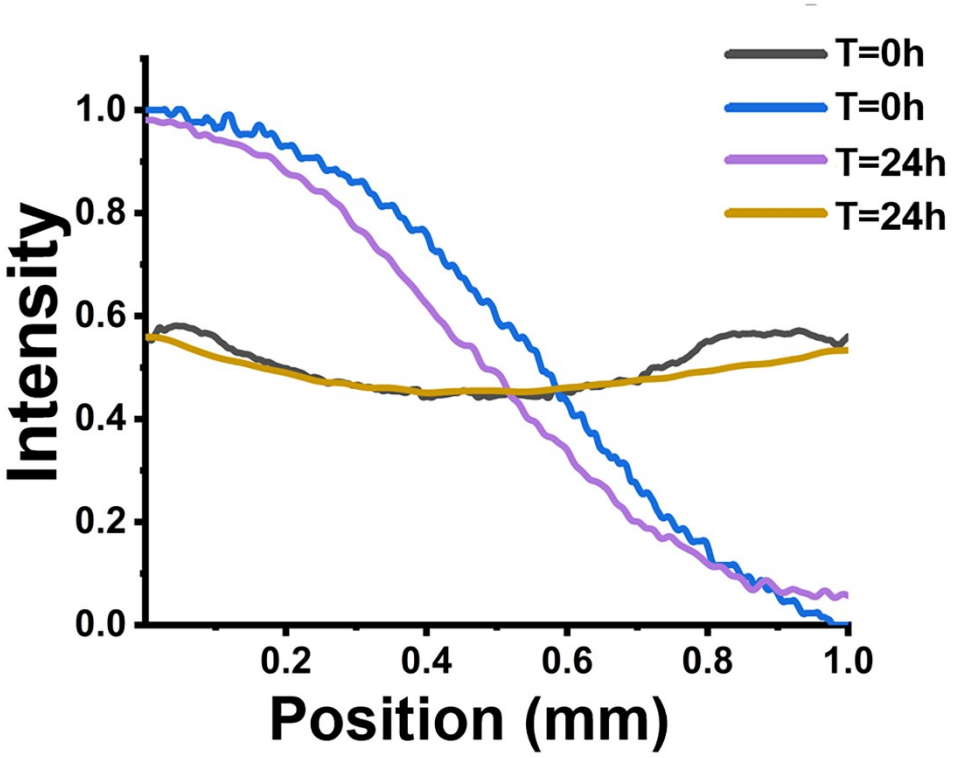

Fig. S6. Linear or nonlinear distribution of RGD peptide concentration at different time points.

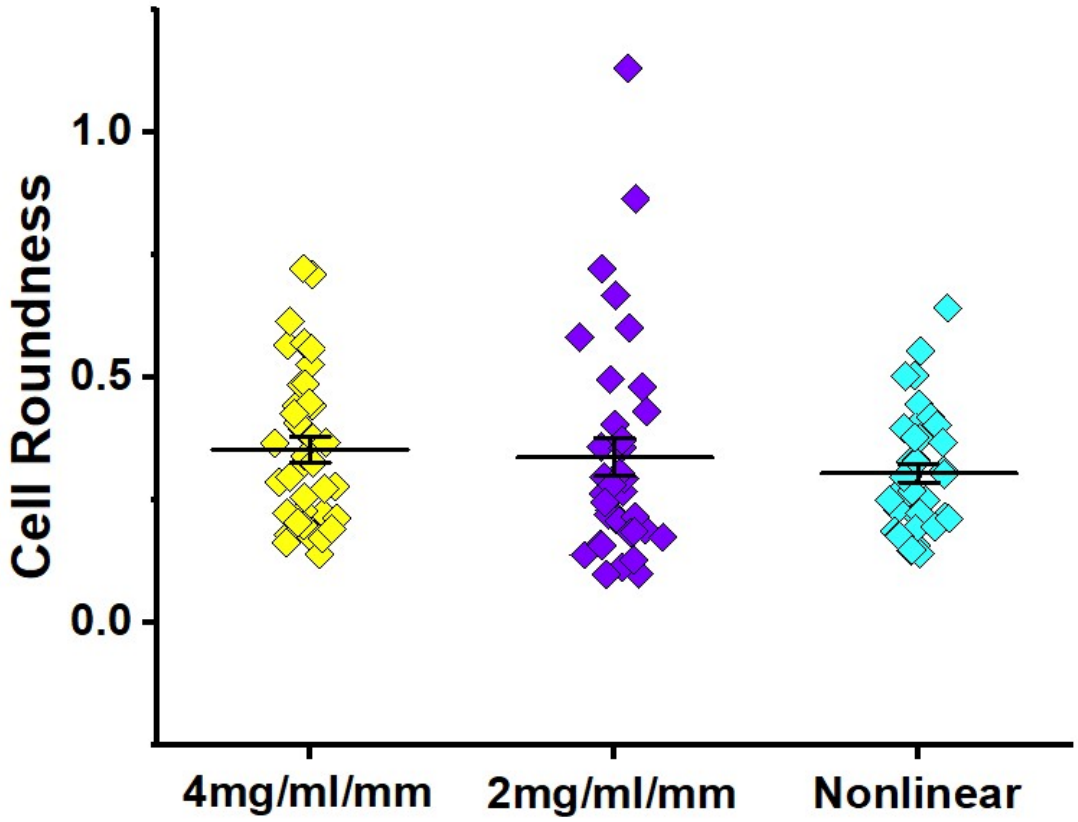

Fig. S7. Quantification of endothelial cell roundness in the different RGD peptide concentration gradients. Data analysis was conducted using a two-sided student's t-test. Data are presented as means ± SEM, n=50-60, from 5-6 independent microfluidic chips.

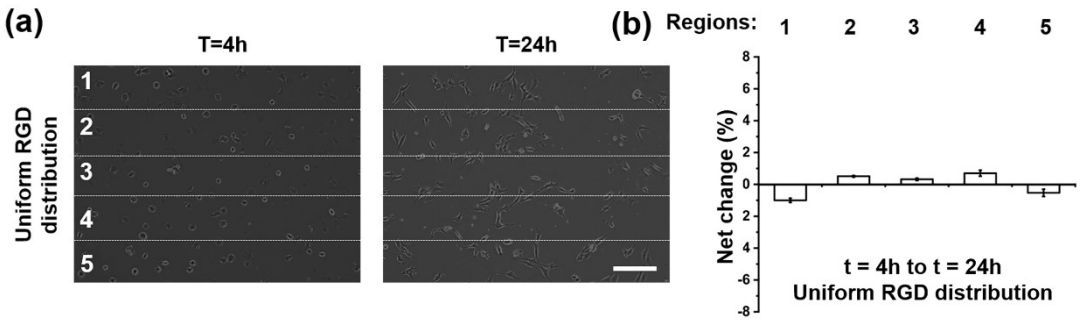

Fig. S8. (a) Representative bright field image of cell position at t=24 h under RGD uniform distribution. (b) Net change rate of cell number in each region under the uniform distribution of RGD using the assess of the cell migration ability. Data represents the mean ± standard deviation from n=3 independent experiments.

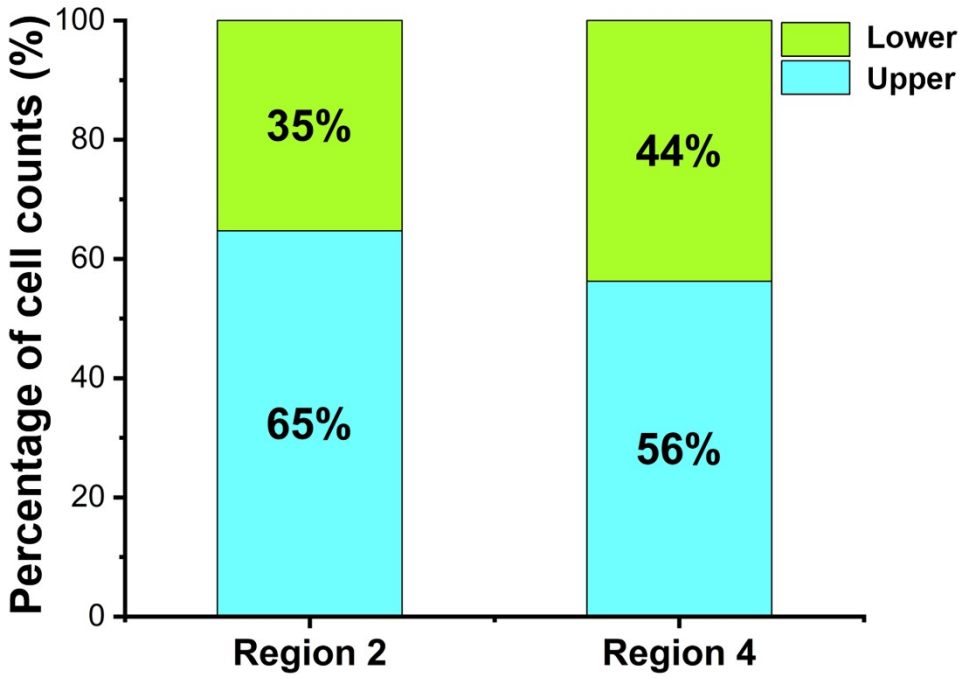

Fig. S9. Percentage of cell counts in areas with upper (closer to designated regions 1/2 and 4/5) or lower (closer to designated regions 2/3 and 3/4) RGD concentrations in regions 2 and 4 at 24h. Data represents the mean deviation from n=3 independent experiments.

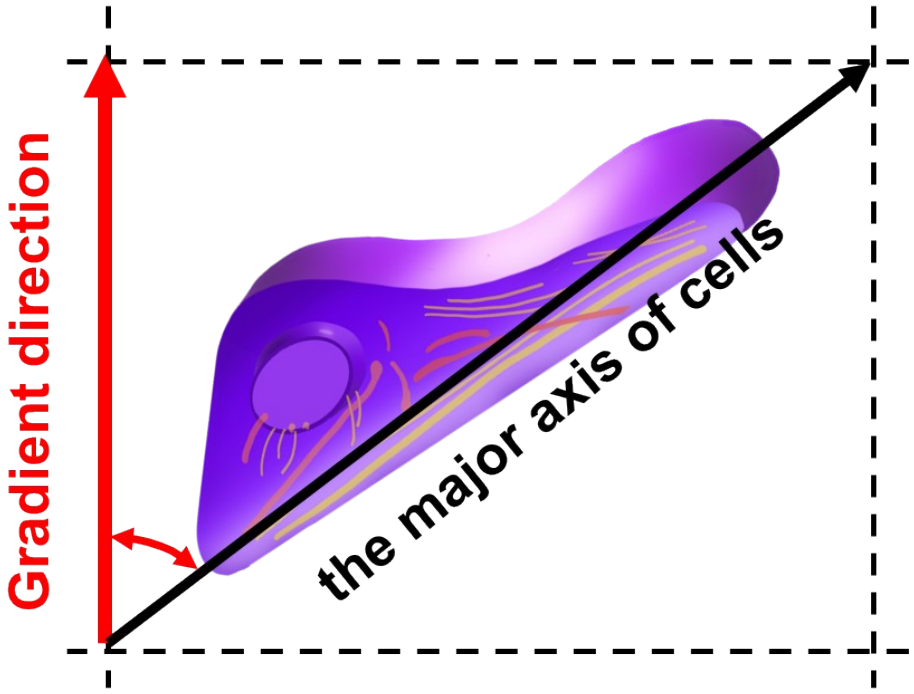

Fig. S10. Schematic diagram for measuring the angle between the major axis of endothelial cells and the concentration gradient.

```
clc; clear; close all;
thisScreenSize = get(0, 'ScreenSize');
[file, path] = uigetfile({'*.png';'*.tif';'*.*'}, 'select a picture');
if isequal(file, 0)
    disp('failed');
else
    disp(['got file from ', fullfile(path, file)]);
end
fullfile = strcat(path, file);
im = imread(fullfile);
figure, imagesc(im), colormap(gray), title("orignal image");
imGray = im2gray(im);
figure, imagesc(imGray), colormap(gray), title("gray image");
imAdapt = adapthisteq(im);
figure, imagesc(imAdapt), colormap(gray), title("startPage image");
prompt = {'insert around cell numbers in this pictureï¼ˆdefault = 30ï¼?'};
title_name = '';
numlines = 1;
defaultanswer = {'30'};
answer = inputdlg(prompt,title_name,numlines,defaultanswer);
N = sscanf(answer{1},'%lf');
figure,
set(gcf,'position',[0, 0, thisScreenSize(3), thisScreenSize(4)])
imagesc(imAdapt), colormap(gray), title("mark from startPage image");
hold on
Angle = zeros(1, N);
for k = 1 : N
    [x, y] = ginput(2);
    x = round(x);
    y = round(y);
    plot(x, y,'+','MarkerSize', 4,'MarkerFaceColor','m','MarkerEdgeColor','g','LineWidth',1);
    for j = min(x) : max(x)
        plot(j, y,'.','MarkerSize', 1,'MarkerFaceColor','m','MarkerEdgeColor','b','LineWidth',1);
    end
    for i = min(y) : max(y)
        plot(x, i,'.','MarkerSize', 1,'MarkerFaceColor','m','MarkerEdgeColor','b','LineWidth',1);
    end
    [n, m] =size(im);
    Slope = ((n - y(1)) - (n - y(2)))/(x(1) - x(2));
    Angle(k) = atan(Slope) * 180/pi;
    str = [num2str(k),', ', num2str(round(Angle(k)))];
    text(round(mean(x)), round(mean(y)), str, 'Color', 'red', 'FontSize', 8, 'FontWeight', 'bold');
end
hold off
```

```
saveas(gca, [path, 'FigWithText.png']);
figure, stem(sort(Angle),'LineStyle','-.','MarkerFaceColor','red','MarkerEdgeColor','green');
ylabel('angle of cell')
ylim([-90, 90])
state = xlswrite([path, 'Angle.xlsx'], Angle', 'sheet1', 'A1');
```

Fig. S11. Code for measuring the angle between the major axis of endothelial cells and concentration gradient using MATLAB

3. References

1 J. H. He, Q. Liu, S. Zheng, R. J. Shen, X. L. Wang, J. M. Gao, Q. S. Wang, J. L. Huang and J. D. Ding, *ACS Appl Materials Inter.*, 2021, **13**, 42344-42356.