

Supporting Information

Effects of microstripe geometry on guided cell migration

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Supplementary Materials include 17 figures, 13 tables and 3 movies. In this file, the figures and tables are presented in the sequence of their citations in the main manuscript. The movies are shown as three separate video files.

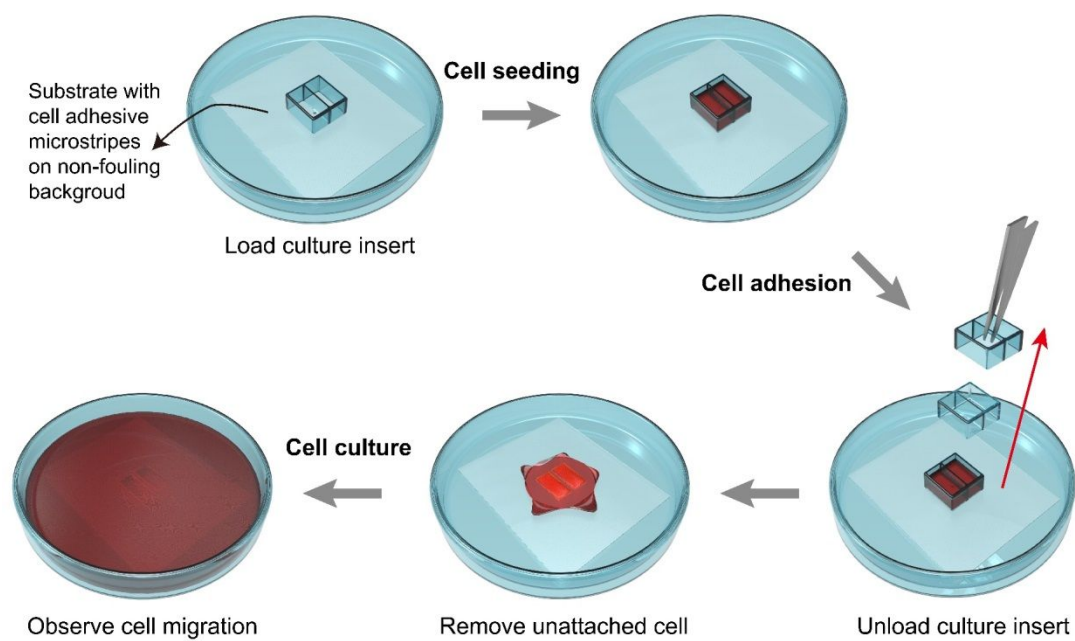


Figure S1. Schematic presentation of the procedure to observe the microstripe-guided cell migration by using a surface patterning technique and a cell culture insert.

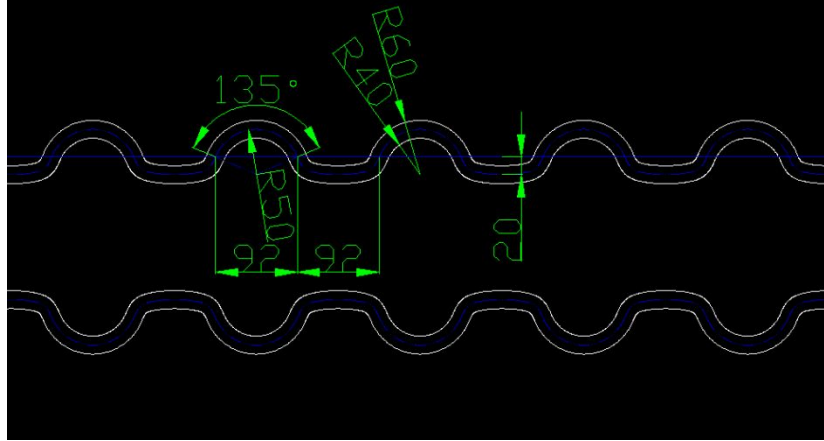


Figure S2. Original design drawing of the combinatory microstripe R50. The white lines here are the corresponding edges of combinatory microstripe R50, the green lines here are the auxiliary lines for the pattern designing with the indicated radiuses (R) and lengths in units of micrometer.

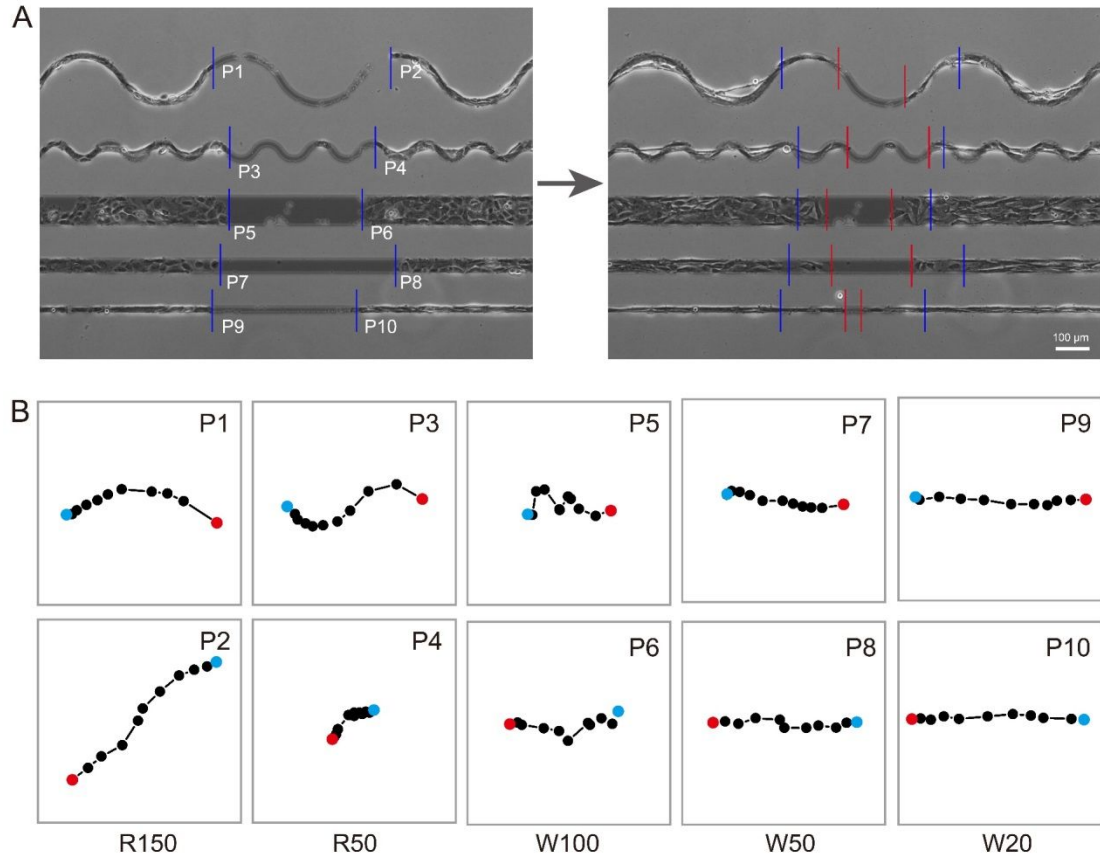


Figure S3. Cell migration along the typical straight and wavy microstripes in one view field in an optical microscope. (A) Phase contrast micrograph to show the relative positions of cell clusters (P1 – P10) at the initial and final states for Movie S1. The blue and red vertical lines indicate the corresponding initial and final positions of the fronts of the cell clusters, respectively. (B) Trajectories of the fronts of the indicated cell clusters during 5.8 h. Each site represents a time-dependent position of the cell fronts; the blue and red dots indicate the corresponding initial and final site, respectively. Each square in (B) has side length of about 280 μm .

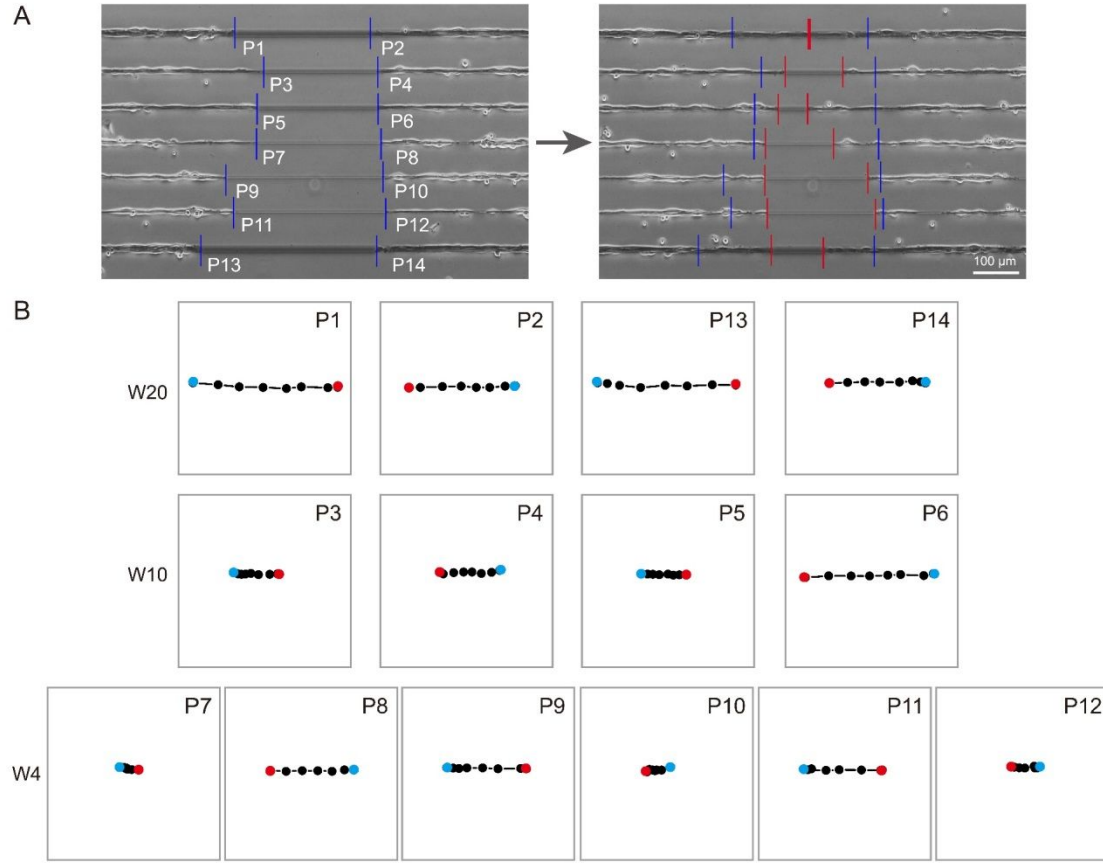


Figure S4. Cell migration along the micropatterns in another view field in our optical microscope to demonstrate typical cases of straight microstripes of widths equal to or less than 20 μm . (A) Phase contrast micrograph to show the relative positions of cell clusters (P1 – P14) at the initial and final states for Movie S2. The blue and red vertical lines indicate the corresponding initial and final positions of the fronts of the cell clusters, respectively. In this independent experiment, the gap distance (about 300 μm) is smaller than that in a regular experiment (about 500 μm). So, we only show the 4 h data for the gap region here, while the statistical results of migration velocity in other figures are still from the 6 h data from other ROI (side regions), as shown at the top-left corner of Figure 2. (B) Trajectories of the fronts of the indicated cell clusters during 4.1 h. Each square in (B) has side length of about 200 μm .

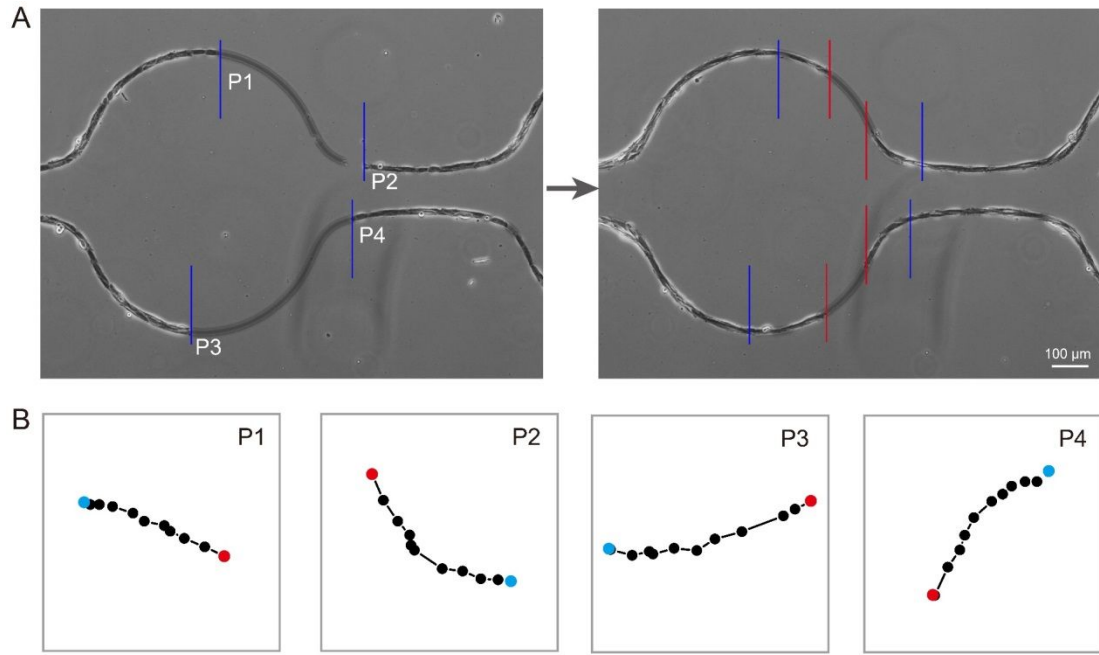


Figure S5. Cell migration along the combinatory microstripes of R400 in one view field in our optical microscope. (A) Phase contrast micrograph to show the relative positions of cell clusters (P1 – P4) at the initial and final states for Movie S3. The blue and red vertical lines indicate the corresponding initial and final positions of the fronts of the cell clusters, respectively. (B) Trajectories of the fronts of the indicated cell clusters during 5.8 h. Each square in (B) has side length of about 300 μm .

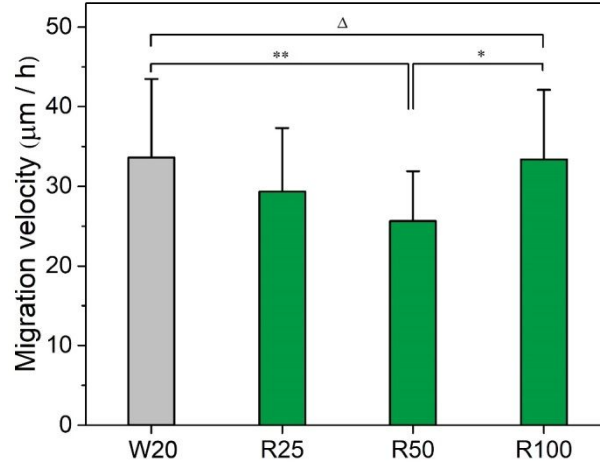


Figure S6. Statistical results of migration velocities of rMSCs on wavy microstripes with arc radiuses of 25 μm (R25), 50 μm (R50) and 100 μm (R100) during the initial 6 h. Here the group of the straight microstripe with width of 20 μm (W20) is as control. The widths of wavy microstripes are all 20 μm and the indicated R's are actually the arc radiuses of the middle arcs of the corresponding wavy stripes. The symbols indicate the significance or insignificance of difference between two indicated groups. “ Δ ”: $p > 0.05$; “*”: $p < 0.05$; “***”: $p < 0.01$.

Table S1. The p values of one-way ANOVA of the data in Figure 3A for migration of rMSCs on straight microstripes with different widths.

Groups	W4	W10	W20	W50	W100
W4	--	9.7E-7	3.3E-10	0.85	0.02
	--	***	***	Δ	*
W10	9.7E-7	--	0.34	0.003	4.0E-8
	***	--	Δ	**	***
W20	3.3E-10	0.34	--	2.1E-4	3.2E-10
	***	Δ	--	***	***
W50	0.85	0.003	2.1E-4	--	0.04
	Δ	**	***	--	*
W100	0.02	4.0E-8	3.3E-10	0.04	--
	*	***	***	*	--

The p value of a global test among W4, W10 and W20 is 1.2E-9 (***).

The p value of a global test among W20, W50 and W100 is 1.8E-12 (***).

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“***”: $p < 0.01$, significant difference; “****”: $p < 0.001$, very significant difference.

Table S2. The p values of one-way ANOVA of the data in Figure 3B for migration of rMSCs on W20 and wavy microstrips with different arc radiuses.

Groups	W20	R50	R150
W20	--	0.008	0.20
	--	**	Δ
R50	0.008	--	0.003
	**	--	**
R150	0.20	0.003	--
	Δ	**	--

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“***”: $p < 0.01$, significant difference; “****”: $p < 0.001$, very significant difference.

Table S3. The p values of one-way ANOVA of the data in Figure 3C for migration of rMSCs on W20 and combinatory microstrips with different arc radiuses.

W20	R50	R150	R400
L-R vs R-L	CCW vs CW	CCW vs CW	CCW vs CW
0.98	0.44	0.15	0.04
Δ	Δ	Δ	*

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“***”: $p < 0.01$, significant difference; “****”: $p < 0.001$, very significant difference.

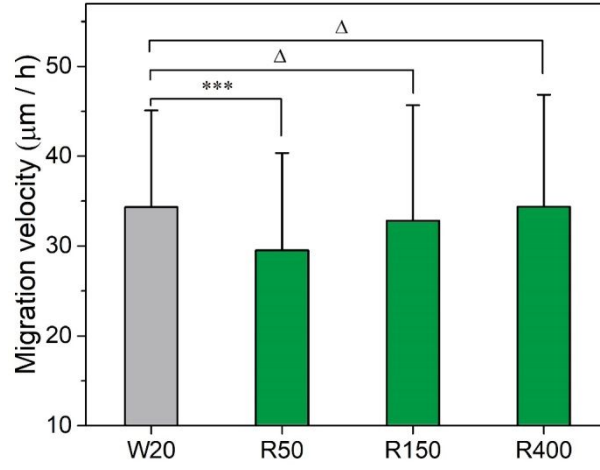


Figure S7. Statistical results of migration velocities of rMSCs on combinatory microstripes of varied arc radiuses during the initial 6 h. CW migration and CCW migration were not distinguished here. The symbols indicate the significance or insignificance of difference between two indicated groups. “ Δ ”: $p > 0.05$; “***”: $p < 0.001$.

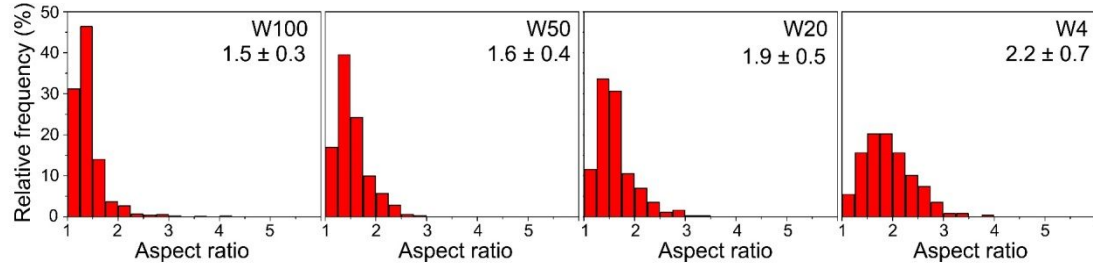


Figure S8. Statistical results of nuclear aspect ratios of rMSCs after 5 h of cell seeding on the indicated straight microstripes. Data on the top-right corner are mean \pm SD of the corresponding aspect ratios of cell nuclei.

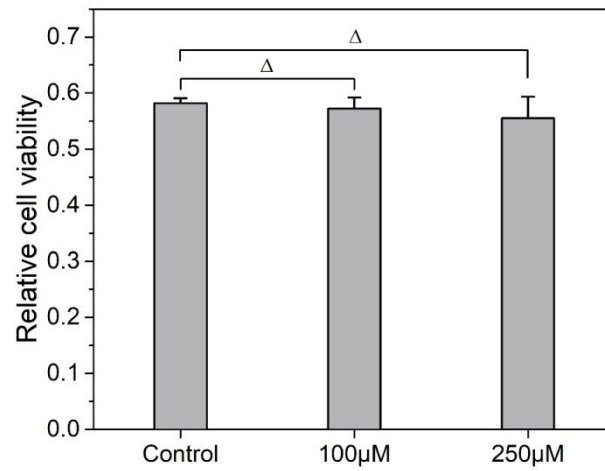


Figure S9. Relative cell viability of rMSCs cultured on tissue culture plates (TCPs) without or with a gap-junction inhibitor Gap-27 of the indicated concentrations. The symbols indicate the significance or insignificance of difference between two indicated groups. “ Δ ”: $p > 0.05$.

Table S4. The p values of one-way ANOVA of the data in Figure 5B for migration of rMSCs on combinatory microstripes and W20 in presence of Gap-27.

W20	R50	R150	R400
L-R vs R-L	CCW vs CW	CCW vs CW	CCW vs CW
0.72	0.31	0.94	0.91
Δ	Δ	Δ	Δ

“ Δ ”: $p > 0.05$, no significant difference

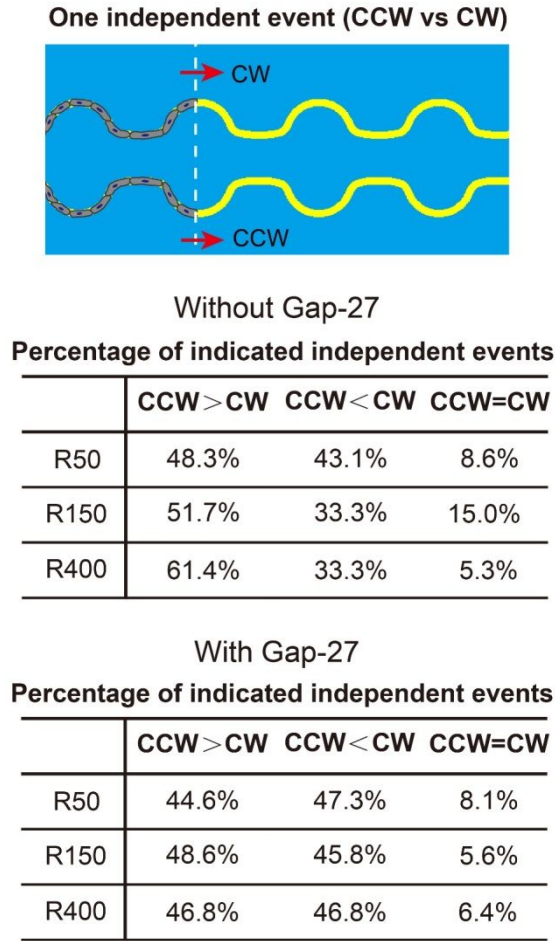


Figure S10. Comparison between CW and CCW events of migration of rMSCs on paired combinatorial microstripes during the initial 6 h trying a modified range of criteria. Schematic presentation of one independent event for the comparison of CW and CCW migrations (two adjacent combinatorial microstripes with the same arc radius) and corresponding statistical results of fractions of independent events under the conditions without or with Gap-27. Here, we have modified the evaluation criteria of “CCW = CW” as $95\% \times CW \leq CCW \leq 105\% \times CW$, different from $99\% \times CW \leq CCW \leq 101\% \times CW$ in Figure 3D in the main manuscript. On each pair of microstripes as seen in the above image, an independent cellular event was defined via comparison between a CW migration on one microstripe and a CCW migration on its adjacent microstripe: a cellular event was regarded as “CCW > CW” if the migration velocity of CCW be 1.05 times higher than that of CW; if the migration velocity of CCW be 0.95 times lower than that of CW, this independent event was marked as “CCW < CW”. The modification of the criteria range from (99%, 101%) to (95%, 105%) was confirmed not to alter the conclusion about migration chirality under the same experimental condition.

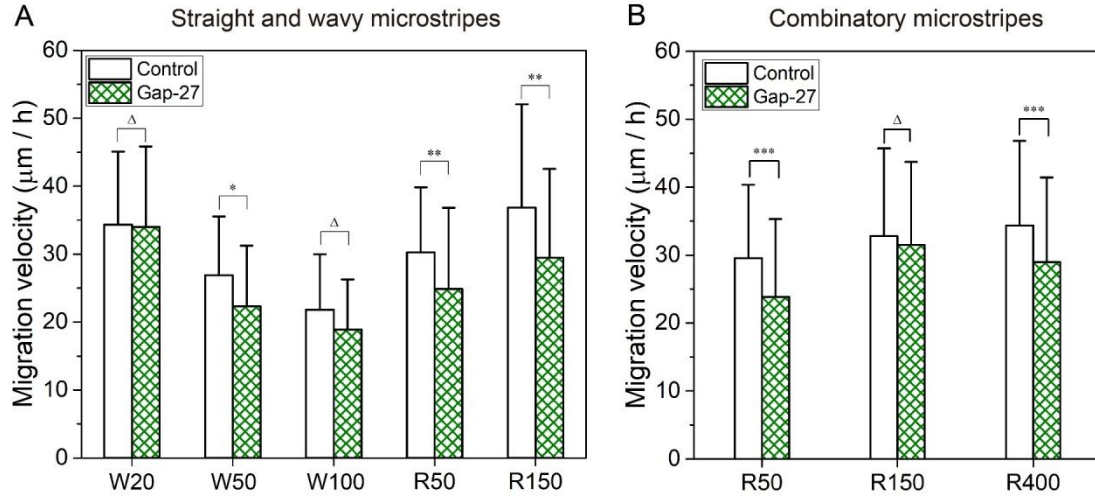


Figure S11. Migration velocities of rMSCs on typical microstripes with or without Gap-27 during the initial 6 h. (A) Statistical results of migration velocities on typical straight (W20, W50, W100) and wavy (R50, R150) microstripes. (B) Statistical results of migration velocities on combinatory microstripes (R50, R150, R400). CW migration and CCW migration on the combinatory microstripes were not distinguished here. The symbols indicate the significance or insignificance of difference between two indicated groups. “Δ”: $p > 0.05$; “*”: $p < 0.05$; “**”: $p < 0.01$; “***”: $p < 0.001$.

Table S5. The p values of one-way ANOVA of the data in Figure 6A for migration of NIH3T3 cells on straight microstripes with different widths.

Groups	W4	W10	W20	W50	W100
W4	--	1.6E-4	5.4E-8	0.15	0.07
	--	***	***	Δ	Δ
W10	1.6E-4	--	0.07	7.8E-5	2.3E-5
	***	--	Δ	***	***
W20	5.4E-8	0.07	--	2.5E-7	6.7E-8
	***	Δ	--	***	***
W50	0.15	7.8E-5	2.5E-7	--	0.74
	Δ	***	***	--	Δ
W100	0.07	2.3E-5	6.7E-8	0.74	--
	Δ	***	***	Δ	--

The p value of global test among W4, W10 and W20 is 6.125E-7 (***).

The p value of global test among W20, W50 and W100 is 1.743E-11 (***).

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“***”: $p < 0.01$, significant difference; “****”: $p < 0.001$, very significant difference.

Table S6. The p values of one-way ANOVA of the data in Figure 6A for migration of Hela cells on straight microstripes with different widths.

Groups	W4	W10	W20	W50	W100
W4	--	1.0E-4	1.2E-4	0.33	0.67
	--	***	***	Δ	Δ
W10	1.0E-4	--	0.89	0.02	0.004
	***	--	Δ	*	**
W20	1.2E-4	0.89	--	0.03	0.005
	***	Δ	--	*	**
W50	0.33	0.02	0.03	--	0.61
	Δ	*	*	--	Δ
W100	0.69	0.004	0.005	0.61	--
	Δ	**	**	Δ	--

The p value of global test among W4, W10 and W20 is 1.2E-4 (***).

The p value of global test among W20, W50 and W100 is 0.005 (**).

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“**”: $p < 0.01$, significant difference; “***”: $p < 0.001$, very significant difference.

Table S7. The p values of one-way ANOVA of the data in Figure 6A for migration of NIH3T3 cells on W20 and wavy microstripes with different arc radiuses.

Groups	W20	R50	R150
W20	--	0.007	0.17
	--	**	Δ
R50	0.007	--	0.26
	**	--	Δ
R150	0.17	0.26	--
	Δ	Δ	--

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“**”: $p < 0.01$, significant difference; “***”: $p < 0.001$, very significant difference.

Table S8. The p values of one-way ANOVA of the data in Figure 6A for migration of Hela cells on W20 and wavy microstripes with different arc radius.

Groups	W20	R50	R150
W20	--	0.002	1.0
	--	**	Δ
R50	0.002	--	0.01
	**	--	*
R150	1.0	0.01	--
	Δ	*	--

“ Δ ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“**”: $p < 0.01$, significant difference; “***”: $p < 0.001$, very significant difference.

Table S9. The p values of one-way ANOVA of the data in Figure 6B for migration of NIH3T3 cells on W20 and combinatory microstripes with different arc radiuses.

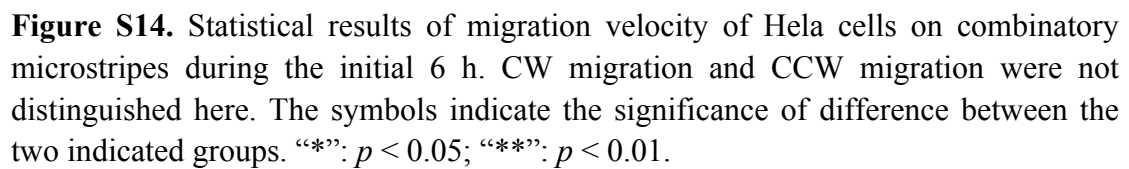
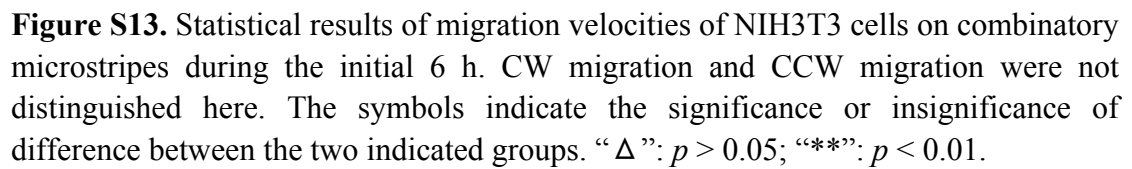
W20	R50	R150	R400
L-R vs R-L	CCW vs CW	CCW vs CW	CCW vs CW
0.99	0.83	0.88	0.30
Δ	Δ	Δ	Δ

“ Δ ”: $p > 0.05$, no significant difference.

Table S10. The p values of one-way ANOVA of the data in Figure 6B for migration of Hela cells on W20 and combinatory microstripes with different arc radiuses.

W20	R50	R150	R400
L-R vs R-L	CCW vs CW	CCW vs CW	CCW vs CW
0.75	0.17	0.36	0.87
Δ	Δ	Δ	Δ

“ Δ ”: $p > 0.05$, no significant difference.



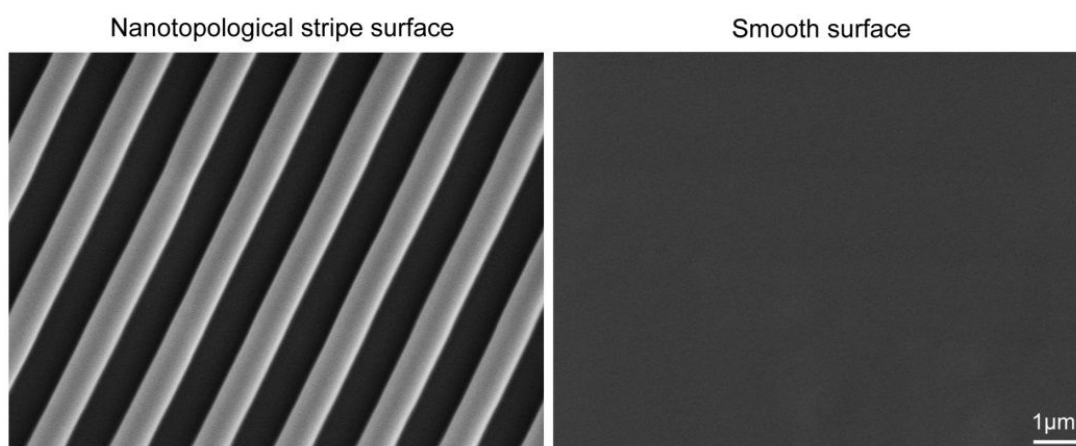


Figure S15. SEM micrographs of petri dishes with a nanotopological stripe surface and a pattern-free smooth surface.

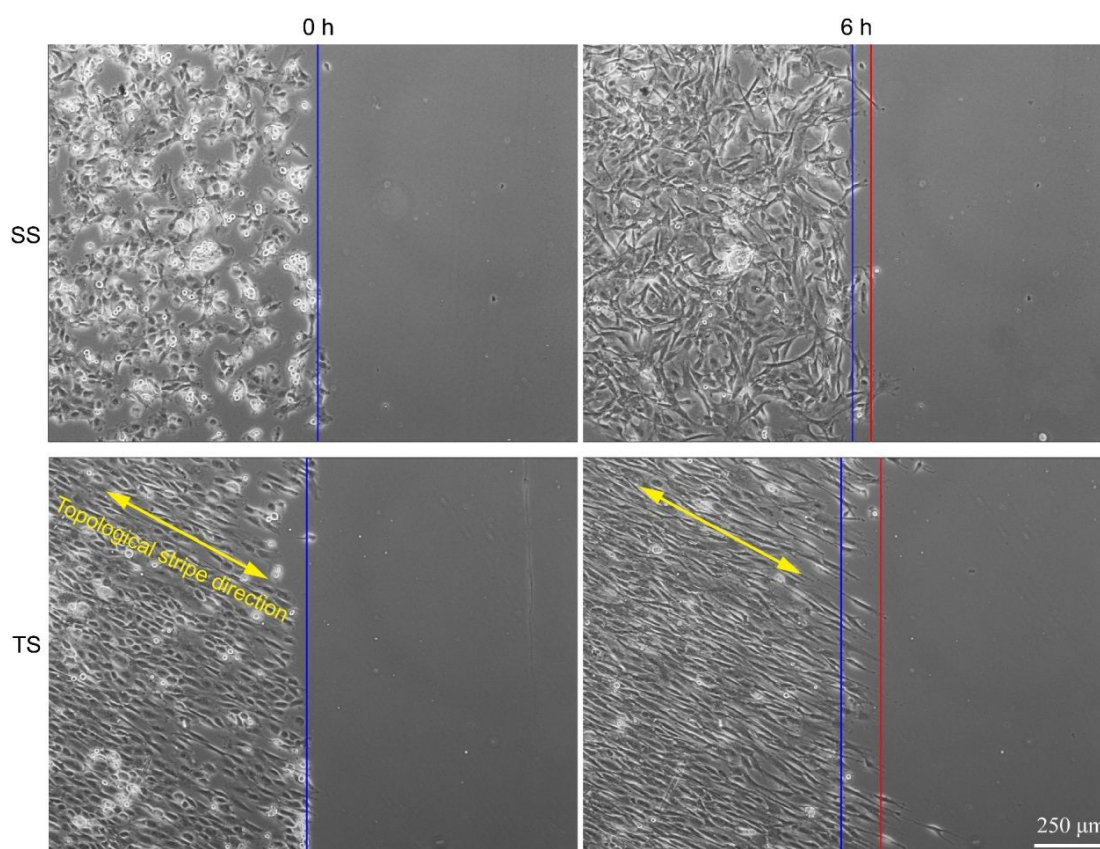


Figure S16. Phase contrast micrographs of migration of rMSCs on nanotopological stripe surface (TS) and smooth surface (SS). “0 h”: initial state of the observation of cell migration (about 4 h after cell seeding). “6 h”: 6 h after the initial observation. The blue and red lines indicate roughly the positions of the initial and migration fronts, respectively.

Table S11. The p values of one-way ANOVA of the data in Figure 7B for migration of rMSCs on W20, nanotopological stripe and smooth surfaces.

Groups	W20	TS	SS
W20	-- --	4.0E-5 ***	3.3E-25 ***
TS	4.0E-5 ***	-- --	1.1E-7 ***
SS	3.3E-25 ***	1.1E-7 ***	-- --

“***”: $p < 0.001$, very significant difference.

Table S12. The p values of one-way ANOVA of the data in Figure 7B for migration of NIH3T3 cells on W20, nanotopological stripe and smooth surfaces.

Groups	W20	TS	SS
W20	-- --	5.0E-7 ***	1.1E-20 ***
TS	5.0E-7 ***	-- --	7.7E-8 ***
SS	1.1E-20 ***	7.7E-8 ***	-- --

“***”: $p < 0.001$, very significant difference.

Table S13. The p values of one-way ANOVA of the data in Figure 7B for migration of Hela cells on W20, nanotopological stripe and smooth surfaces.

Groups	W20	TS	SS
W20	-- --	0.01 *	3.7E-8 ***
TS	0.01 *	-- --	0.02 *
SS	3.7E-8 ***	0.02 *	-- --

“Δ”: $p > 0.05$, no significant difference; “*”: $p < 0.05$, significant difference;

“***”: $p < 0.01$, significant difference; “****”: $p < 0.001$, very significant difference.

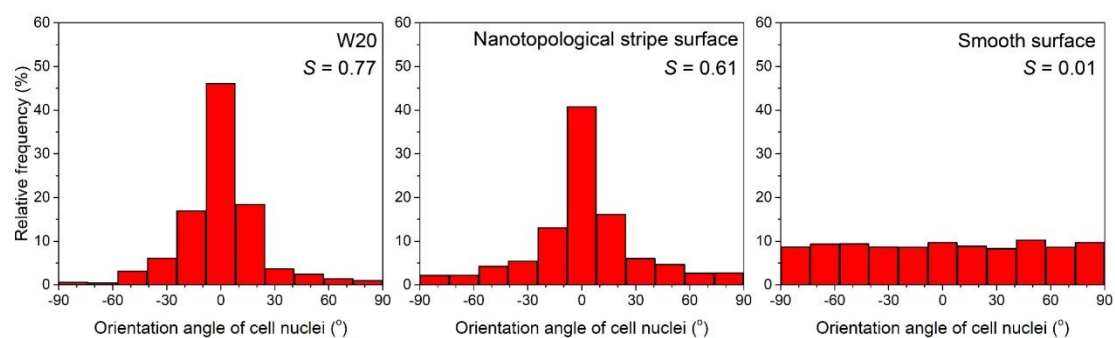


Figure S17. Statistical results of nuclear orientation of Hela cells after 5 h of cell seeding on the indicated surfaces. W20: straight microstripe with width of 20 μm . S refers to order parameter.