Chapter IV: Scaling Micropattern Dimensions to Enable and Modulate Directed Cell Movement

1. Abstract

Micron-scale geometrical constraints shape cell morphology and affect cell motility. However, cell types differ in their response to geometric cues. Elucidating the underlying factors could instruct how to redesign micron-scale features to induce desired migratory properties in recalcitrant cell types. Here, we show that directional bias in cell movement on teardrop-based micropatterns is highly correlated to the establishment of a unilamellar morphology in fibroblasts, keratinocytes and mammary epithelial cells. Furthermore, narrowing the width of teardrop micropatterns enhances the establishment of a unilamellar morphology and increases the directional bias of movement of normal human epidermal keratinocytes (NHEK). These thin teardrops increase the bias of MCF-10A epithelial cells as well, but unexpectedly create a moderate bias on a previously unbiased configuration. These results give us insight into how cells can respond to different degrees of geometrical constraints (i.e., what cells interpret as tip as opposed to blunt) and how such constraints at the ends of the island dictate directional movement of cells on micropatterns. These findings underscore the importance of a unilamellar morphology in achieving directed cell migration on micropatterns and offer design strategies to promote directional bias in migration of different cell types for tissue engineering applications.

2. Introduction

Directional cell migration involves the establishment of front-rear (FR) polarity.[1, 2] The front is typically a broad lamella. Meanwhile, the narrow trailing end is more sensitive to myosin-generated contractile forces, facilitating the release of adhesions in the rear and net forward cell movement. The stability of FR polarity during cell migration is transient and helps in determining the persistence of the random walk in an isotropic microenvironment.

Micropatterns can influence the symmetry breaking process needed to establish and maintain FR polarity. For example, fibroblasts on extremely thin (1.5 μm) adhesive line patterns can break symmetry and assume a motile uniaxial morphology with a single lamella. [3] Furthermore, we have shown with MCF-10A epithelial cells that the spatial constraints imposed by micropatterned lines (20 μm width) forces single lamella that are narrower and more stable, leading to greater persistence in migration than observed on uniform substrates (Chapter III). Thus, the narrower, more stable lamella establishes and maintains a sharpened FR polarity and a distinct unilamellar morphology. Symmetry breaking is not unqiue to line patterns. Two cells occupying a circular island can break symmetry and start rotating in the same direction. Their yin-yang morphology is akin to the unilamellar morphology as well.[4]

Micropattern geometry can also give directional cues to cells. For example, Co and colleagues found that directional movement of 3T3 fibroblasts can be induced using teardrop-based micropatterns.[5] However, different cell lines can respond differently to

similar micropattern geometry. When a similar geometrical constraint was applied on MCF-10A epithelial cells, the movement bias was in the opposite direction.[6]

In this study, we sought to better understand how different cell lines interpret the underlying geometrical constraints and look for a universal predictor for directional cell movement. Perhaps, the establishment of the unilamellar morphology, which in itself represents directional orientation, is important in determining directional bias on these micropatterns. Furthermore, understanding the role of unilamellar morphology in directional cell movement on micropatterns may lead to more general strategies to modulate and enhance directional bias for all cell types.

3. Results

3.1. Cell types differ in the extent of directional bias on teardrop patterns

We previously showed that MCF-10A cells exhibit a high bias in movement on square migration tracks composed of teardrop-shaped micropatterns. [6] The cells traverse the track by hopping from one adhesive island to the next. A high bias is exhibited for hopping sideways from a tip to a blunt end (sT>B hop) when the teardrop patterns are arranged in Configuration A. No directional bias is observed in Configuration B, a track that lacks a junction for a sT>B hop (**Figure 1A**). These and other results demonstrated that the directional bias of MCF-10A cell movement stems from lamellipodial activity that extends preferentially sideways (not head-on) out of the tip ends of teardrop patterns. Enhancing lamellipodial stability by moderately reducing

the expression of Rac1 using siRNA interference enabled head-on lamellipodial extensions and flipped the directional bias of MCF-10A cell movement.

To test the generality of using teardrop micropatterns to direct cell migration, we examined the movement of other cell lines on the same teardrop-shaped micropatterns. Directional bias was quantified as the fraction of successful jumps in either the tip-to-blunt (T>B) or blunt-to-tip (B>T) direction. Normal human epidermal keratinocytes (NHEK) show a moderate bias on Configuration A with 66% of hops in the sT>B direction (**Figure 1B**, Supplementary Data **Movie 1**). While the preferred direction matches that of MCF-10A cells, the bias is quantitatively weaker in NHEKs. Similar to MCF-10A cells, NHEKs exhibit little to no bias on Configuration B (Supplementary Data **Movie 2**). Lastly, Rat1 fibroblasts show little to no bias on both configurations of teardrop patterns (**Figure 1C**, Supplementary Data **Movie 3)**. These results show that cell types differ significantly in the extent of directional bias, although where a bias is exhibited (moderate in NHEK and strong in 10A cells), the direction of cell movement consistently favors a sideway tip-to-blunt hop.

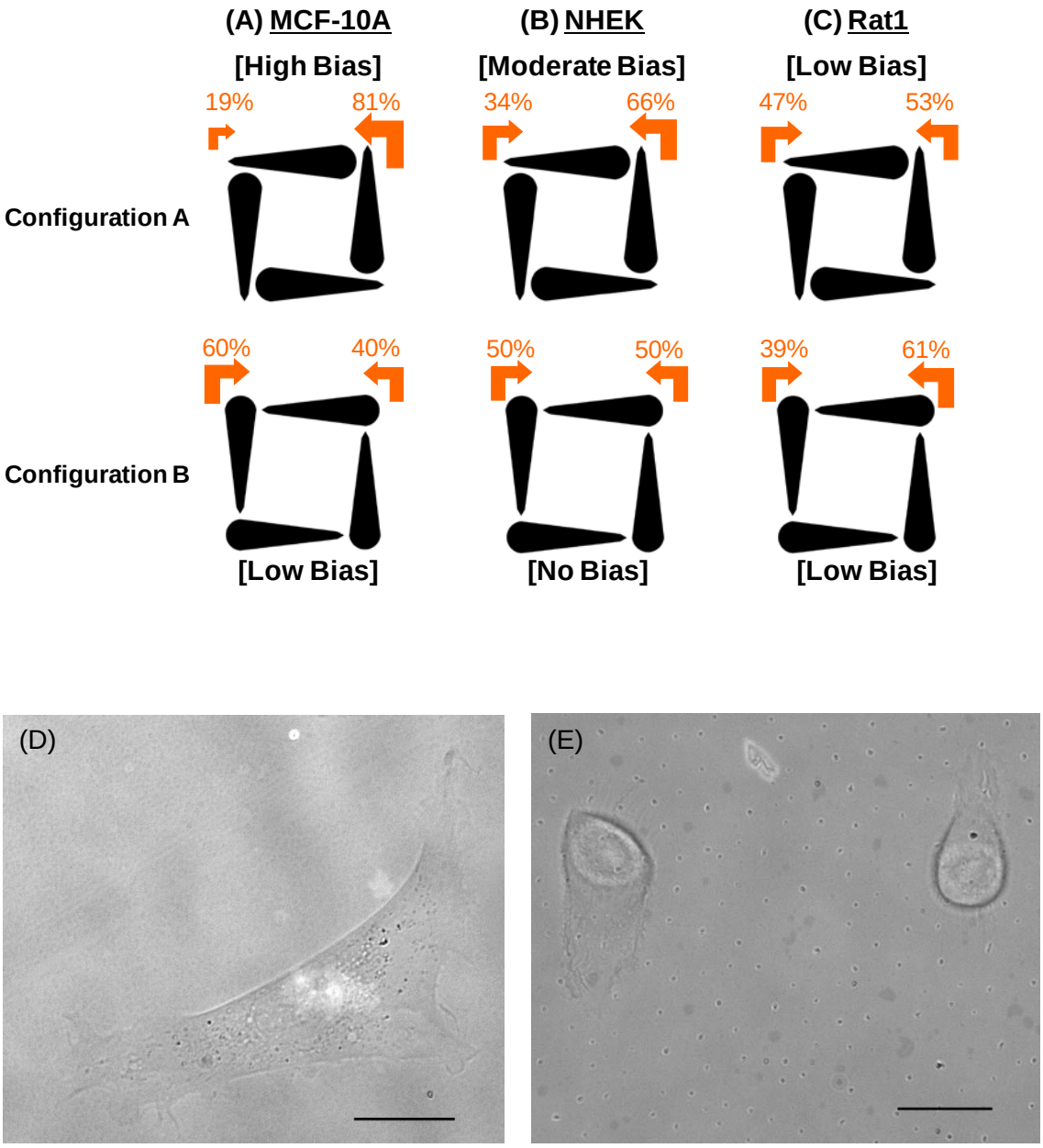

Figure 1. Motility biases for (A) MCF-10A epithelial cells, (B) NHEKs and (C) Rat1 fibroblasts on the original teardrop patterns. On Configuration A, MCF-10A epithelial cells showed high bias in the sideway tip to blunt direction, followed by moderate bias of NHEKs and no bias for Rat fibroblasts. (D) Rat1 fibroblasts on Pattern A do not establish any FR polarity, while (E) most of MCF-10A cells and some NHEKs establish a

strong FR polarity on Configuration A [Scale bar = 20μm]. On Configuration B, the cells showed relatively low to no bias.

3.2. Establishment of unilamellar morphology correlates with extent of directed cell movement on teardrop patterns

Qualitatively, we noticed that these cell types differ in the establishment of FR polarity. Rat1 fibroblasts and some of the NHEKs form lamellipodial fronts on two ends (i.e., no polarity), resulting in a tug-of-war between the two fronts and preventing directional movement (**Figure 1D**). On the other hand, almost all of the MCF-10A cells and many of the NHEKs establish a stable FR polarity with a single prominent lamella (**Figure 1E**),[6] closely resembling the unilamellar morphology observed in other studies.[3, 4] These observations suggested that the inability to establish or maintain unilamellar morphology may impair directional movement of NHEKs and the fibroblasts.

To probe more deeply the relationship between the unilamellar morphology and directed cell movement on teardrop patterns, we needed a technique to modulate the ability of cells to acquire the unilamellar morphology. Since unilamellar morphology was observed previously on extremely thin line patterns,[3] we hypothesized that the establishment of an unilamellar morphology may correlate with and be tuned by the width of the micropattern.

To test this idea, we plated the three cell types on micropatterned lines of different widths ranging from 5 - 20 μm and quantified the occurrence of unilamellar morphology.

We found that the line width affects the fraction of cells exhibiting the unilamellar morphology (**Table 1**). Almost all (92%+) of the MCF-10A cells become unilamellar within a 12-hour period on 20 micron lines; reducing the line width did not significantly enhance this saturated ability to attain a unilamellar morphology in 10A cells. Meanwhile, only 61% of the NHEKs become unilamellar on the 20 μm lines. This fraction increases significantly to 86% on thinner lines (5 and 10 μm). Almost none (<1%) of the Rat1 fibroblasts assume the unilamellar morphology on all line widths, consistent with the previous report that 3T3 fibroblasts exhibit a unilamellar morphology only on thin lines below 5 μm widths and predominantly on extremely thin lines of 1.5 μm width,[3] a feature size below the working range of our microcontact printing methodology.[7]

These quantitative measurements show that the ability of cells to acquire a unilamellar morphology is greatest for MCF-10A cells, followed by NHEKs and then Rat1 fibroblasts. This tendency to achieve unilamellar morphology correlates with the extent of biased movement on teardrop patterns, suggesting that establishment of such morphology may be critical to achieving directed movement. Furthermore, since narrowing line widths increases the occurrence of unilamellar morphology in NHEK cells, it presents an opportunity to test whether narrowing teardrop patterns may be a design strategy to induce or enhance directional bias in cell migration.

	Non-pattern	Line (5μm)	Line (10μm)	Line (20μm)
MCF-10A (epithelial cells)	N/A (~0%)	96%	96%	92%
NHEK (keratinocytes)	N/A (~0%)	86%	87%	61%
Rat1 (fibroblasts)	N/A (~0%)	1%	0%	0%

Table 1. Tendency to acquire unilamellar morphology for MCF-10A epithelial cells, NHEKs and Rat1 fibroblasts on line patterns of different widths. Fractions of cells that establish unilamellar morphology on the line patterns within the 12 hr-period immediately after seeding are shown. In general, greater constraint (thinner lines) seems to better promote the establishment of stable FR polarity resulting in unilamellar morphology.

3.3. Narrowing teardrop patterns enhances directional bias

To test the idea that narrowing the teardrop patterns may enhance directed cell movement on these micropatterns, we designed teardrop patterns with a maximum width of 10 μm at the blunt end and quantified cell migration on these thinner teardrop patterns (**Figure 2**). Other features of the square track, including the gap distance between teardrops and their relative positioning, were unchanged. The directional bias of NHEKs improved from 66% to 77%, a statistically significant ($p<0.05$) increase, on thin teardrops arranged in Configuration A (Supplementary Data **Movie 4**). Thus, increasing the occurrence of unilamellar morphology enhances directional bias of cell movement.

Although a similar increase was observed for Configuration B (50% to 60% in the head-on T>B direction), the cells jumped less frequently compared to Configuration A, and this change in directional bias was not statistically significant.

As a negative control, we tested the movement of Rat1 fibroblasts on the thinner teardrop patterns. Consistent with the fact that reducing line width to 10 μm had no effect on establishing unilamellar morphology in Rat1 fibroblasts, these cells exhibited no enhancement in directed cell movement on narrow teardrop patterns compared to the original teardrop patterns. Finally, MCF-10A cells, serving as positive control, maintained their high directional bias (81% to 86%, though not statistically significant) even on narrow teardrops in Configuration A, consistent with the fact that the fraction of unilamellar morphology remained near 95% on 10 and 20μm lines.

An unexpected observation, however, was the change in the movement of MCF-10A cells on thin teardrops in Configuration B, which offered additional insights into how cells interpret the rescaling of the teardrop pattern. Our quantitative measurements show that 10A cells on thin teardrops in Configuration B begin to mimic that of Configuration A (Supplementary Data **Movie 5**). Cells now actively hop sideways from the 10 μm blunt end onto the adjacent island's tip. This effect is also reflected in the increased frequency of hops on the narrow Configration B compared to the original Configuration B (data not shown). We conclude that at the 10 μm width, MCF-10A cells begin to respond to the blunt end as a tip, leading to the observed bias in sideways blunt-to-tip hops in the new Configuration B.

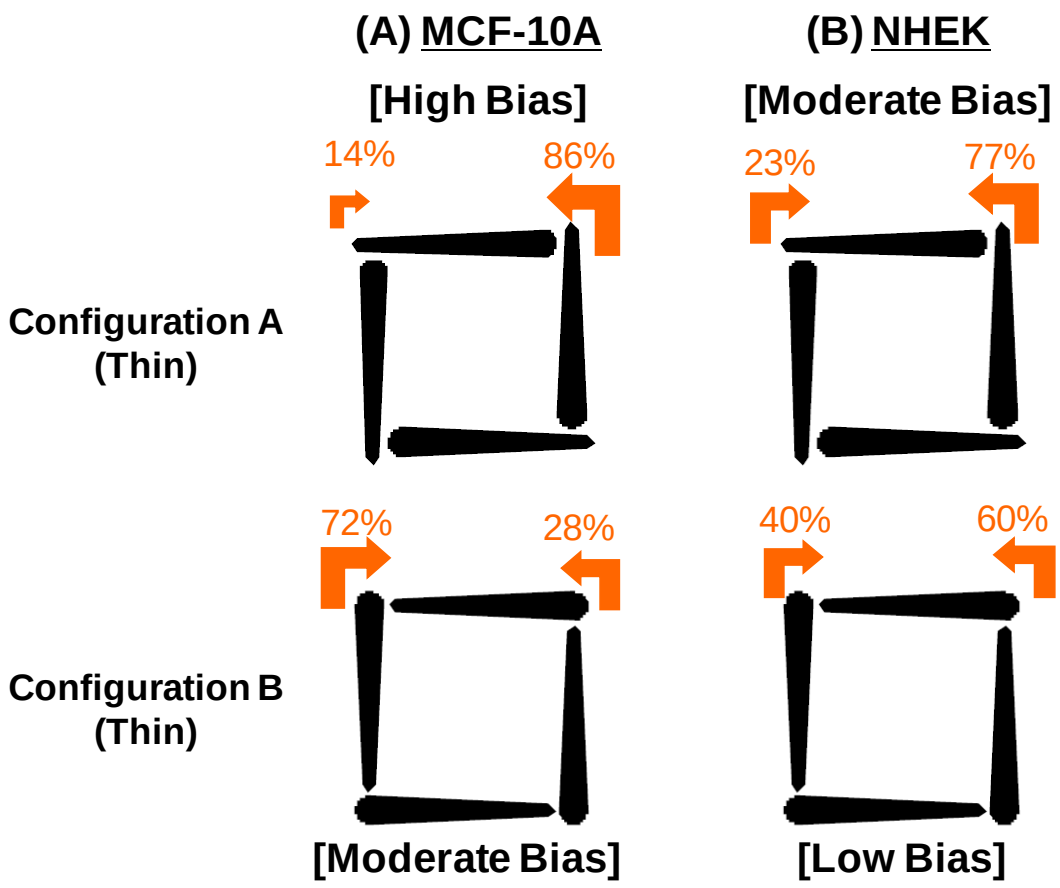

Figure 2. Motility biases for (A) MCF-10A epithelial cells and (B) NHEKs on thin teardrop patterns. On thin Configuration A, both MCF-10A epithelial cells and NHEKs showed significantly increased bias in the sideways tip to blunt direction compared to the original Configuration A. On thin Configuration B, the NHEKs showed relatively low bias, but the MCF-10A cells surprisingly showed a moderate bias in the sideways blunt to tip direction.

It is noteworthy that sideway extensions remain the preferred mode of hopping on the original and thinner teardrops. However, cells on the thin Configuration B exhibit

quantitatively less bias than on the original Configuration A. At least two factors may contribute to this quantitative difference in bias. First, the narrower blunt end (10 μm) is still wider than the tip end in Configuration A. Second, the degree of asymmetry in the narrower teardrop (the ratio of the widths of the blunt and tip end) is less than that presented by the original teardrop.

Taken together, these results demonstrate that the unilamellar morphology plays an important role in directed cell migration on teardrop patterns and show that narrowing teardrop patterns is a strategy to induce unilamellar morphology and enhance directed cell movement. In addition, although the unilamellar morphology is important, it is not sufficient. Approximately 95% of MCF-10A cells acquire unilamellar morphology on 10-20 μm lines but yet fail to exhibit biased movement on the original Configuration B. In addition to the acquisition of a unilamellar morphology, the geometrical constraints of the end from which cells hop are also critical. Adequate physical constraints (in the case of MCF-10A cells, an end constrained to ~10 micron width) must be imposed at a junction where a sideway hop can be executed to an adjacent island.

4. Future Directions

The new observation of increased migratory bias of MCF-10A cells on thin teardrops in Configuration B suggest that the geometric constraints at the ends of the teardrop and a properly positioned target island for a sideway hop may be more important than the asymmetry of the teardrop shape in dictating directional bias on micropatterns. To test this idea, we will parse out the contributions of asymmetry and geometrical

constraint by creating thin rectangles with widths of 5 and 10μm (**Figure 3**). In addition, extra thin teardrops with maximal width of 5μm will be tested on multiple cell lines to induce or enhance directed movement on micropatterns.

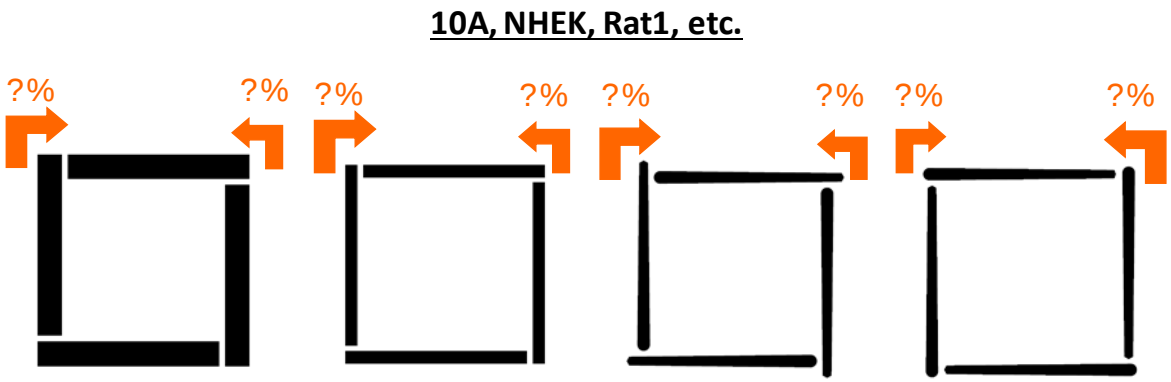

Figure 3. Schematics of thin rectangles and extra thin teardrop patterns to be tested with MCF-10A cells, NHEKs and other cell lines. The thin rectangles are 80μm in length and 10μm or 5μm in width with 3μm gaps. The extra thin teardrops are 80μm in length, 5μm wide at the blunt end and 3μm wide at tip end with 3μm gaps. The thin rectangles will reveal whether asymmetry is need for directional bias, and if so how large the impact is. Extra thin teardrops will be tested in an attempt to induce directional bias for other cell lines (such as fibroblasts), and also to look for similar trends in NHEKs as what we have observed for 10A cells.

5. Conclusion

Cell motility can be governed by the geometrical constraints imposed by the micropattern, but recent studies show that different cell types display different directional bias on similar patterns.[5, 6] In this study, we sought to understand why different cell

types may exhibit different directional bias on similar geometrical patterns, and utilize the findings to develop generalized strategies to control directed motility for different cell types. We compared the directed motility of three cell types on the teardrop-based micropatterns, and found a qualitative correlation between directional bias and their ability to assume unilamellar morphology. Further quantitative analysis using line patterns of different widths revealed that indeed the frequency of unilamellar cells correlates with the geometrical constraint of the lines. In an attempt to increase directional bias for some cell lines, we proceeded to scale-down the teardrop to the width corresponding to maximum frequency of unilamellar morphology and found that indeed the directional bias increases significantly. Furthermore, in addition to the necessity of unilamellar morphology in biased movement, we also found that the degree of geometrical constraints of the ends from which they jump also influences the directional bias. These findings begin to open venues to control the motility and directional bias of different cell types through scaling the dimensions of the patterns.

6. Experimental Methods

6.1. Fabrication of micropatterned substrates

Microcontact printing with a polydimethylsiloxane (PDMS) stamp was used to pattern fibronectin onto a gold-coated chambered coverslide (Labtek), as described previously.[6] Briefly, UV light is passed through a chrome mask containing the pattern (Nanoelectronics Research Facility, UCLA) onto a layer of SU-8 photoresist to make a mold, onto which PDMS is cast to make the final stamp. The stamp is then “inked” with

16-Mercaptohexadecanoic acid (Sigma Aldrich) to print the pattern onto a gold-coated coverslide. The unprinted area is passivated using PEG(6)-Thiol (Prochimia) to prevent non-specific binding of cells. After washing with PBS twice, EDC and Sulfo-NHS (Pierce) is added to the coverslide to activate the acid to crosslink covalently with the amine group of the subsequently added fibronectin (Sigma Aldrich) dissolved in PBS at 10μg/mL. Finally, BSA conjugated with Alexa Fluor 594 (Invitrogen) was doped into the fibronectin solution for the purpose of pattern visualization.

6.2. Cell culture

MCF-10A human epithelial cells were cultured in growth medium composed of Dulbecco's modified Eagle's medium/Ham's F-12 containing HEPES and L-glutamine (DMEM/F12, Invitrogen) supplemented with 5% horse serum (Invitrogen), 1% penicillin/streptomycin, 10 μg/mL insulin (Sigma), 0.5 μg/mL hydrocortizone (Sigma), 20ng/mL EGF (Peprotech) and 0.1 μg/mL cholera toxin (Sigma) and maintained under humidified conditions at 37ºC and 5% CO_2. Cells were passaged regularly by dissociating confluent monolayers with 0.05% trypsin-EDTA (Invitrogen) and suspending cells in DMEM/F12 supplemented with 20% horse serum and 1% penicillin/streptomycin. After two washes, cells were diluted 1:4 and plated in growth medium.

Normal human epidermal keratinocytes (NHEKs) were cultured in keratinocyte growth medium-2 (KGM-2, Lonza) and maintained under conditions at 37ºC and 5% CO_2. Cells were passaged regularly according to instructions provided by Lonza. Briefly,

confluent monolayers were dissociated with trypsin/EDTA (Lonza) and cells suspended in trypsin neutralizing solution (TNS, Lonza). After two washes with HEPES Buffered Saline (Lonza), cells were diluted 1:4 and plated in KGM-2.

Rat1 fibroblasts were cultured in growth medium composed of Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin/streptomycin and maintained under humidified conditions at 37ºC and 5% CO_2. Cells were passaged regularly by dissociating confluent monolayers with 0.05% trypsin-EDTA (Invitrogen) and suspending cells in growth medium. After two washes, cells were diluted 1:10~1:12 and plated in growth medium.

6.3. Timelapse microscopy

Cells were seeded in growth medium for 1 hr onto the micropatterned substrate. After washing to remove non-adherent cells, the culture was incubated with fresh growth medium for 1 hr and imaged at 10x magnification every 5 min for 12 hr. Cells were maintained at 37ºC and 5% CO_2 in a heated chamber with temperature and CO_2 controller (Pecon) during time-lapse imaging. Images and movies were acquired using Axiovert 200M microscope (Carl Zeiss), and Axio Vision LE Rel. 4.7 (Carl Zeiss) was used for image analysis.

6.4. Cell motility quantitation and analysis

For line patterns, the lamellipodial position was tracked using Axio Vision LE Rel. 4.7 and ImageJ software. The tendency to establish FR polarity was determined as the fraction of cells that assume FR polarity at one point or another during the course of a 12-hr experiment. Also, migration speed was obtained as the total distance traveled divided by the total time, and the persistence length was based on switching the direction 180° (not based on whether FR polarity was broken or not).

The directional biases on teardrop-based patterns were obtained as described previously,[6] which are fractions of the complete successful jumps in each direction of the pattern. Degree of bias was arbitrarily assigned as “no bias” (50%), “low bias” (51~65%), “moderate bias” (66%~80%) and “high bias” (81%~).

7. Acknowledgements

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References

[1] D. A. Lauffenburger, A. F. Horwitz, *Cell* **1996**, *84*, 359.

[2] A. J. Ridley, M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons, A. R. Horwitz, *Science* **2003**, *302*, 1704.

[3] A. D. Doyle, F. W. Wang, K. Matsumoto, K. M. Yamada, *J Cell Biol* **2009**, *184*, 481.

[4] S. Huang, C. P. Brangwynne, K. K. Parker, D. E. Ingber, *Cell Motil Cytoskeleton* **2005**, *61*, 201.

[5] G. Kumar, C. C. Ho, C. C. Co, *Advanced Materials* **2007**, *19*, 1084.

[6] K. Kushiro, S. Chang, A. R. Asthagiri, *Advanced Materials* **2010**, n/a. doi: 10.1002/adma.201001619

[7] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D. E. Ingber, *Annu Rev Biomed Eng* **2001**, *3*, 335.