

Guidance of Cell Migration by Substrate Dimension

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ABSTRACT There is increasing evidence to suggest that physical parameters, including substrate rigidity, topography, and cell geometry, play an important role in cell migration. As there are significant differences in cell behavior when cultured in 1D, 2D, or 3D environments, we hypothesize that migrating cells are also able to sense the dimension of the environment as a guidance cue. NIH 3T3 fibroblasts were cultured on micropatterned substrates where the path of migration alternates between 1D lines and 2D rectangles. We found that 3T3 cells had a clear preference to stay on 2D rather than 1D substrates. Cells on 2D surfaces generated stronger traction stress than did those on 1D surfaces, but inhibition of myosin II caused cells to lose their sensitivity to substrate dimension, suggesting that myosin-II-dependent traction forces are the determining factor for dimension sensing. Furthermore, oncogene-transformed fibroblasts are defective in mechanosensing while generating similar traction forces on 1D and 2D surfaces. Dimension sensing may be involved in guiding cell migration for both physiological functions and tissue engineering, and for maintaining normal cells in their home tissue.

INTRODUCTION

Cell migration is essential for many biological processes, including development and wound healing. Migration also plays a key role in cancer metastasis and tissue engineering. The process of cell migration involves tightly regulated cycles of polarization, cytoplasmic protrusion, and adhesion formation and detachment (1) guided by environmental cues. Although early work emphasized the role of chemical gradients, it is becoming evident that physical features of the substrate play an equally important role in guiding cell migration.

The effects of physical cues have been demonstrated in a variety of contexts. It has been known for decades that cells migrate preferentially along grooves on a substrate, a phenomenon referred to as contact guidance (2). Other surface topography, such as pillars, also affect cell shape and migration (3–5). Migrating fibroblasts also respond to substrate rigidity by moving toward stiffer substrates, and to stretching forces by reorienting in the direction of tensile forces (6). By micropatterning adhesion areas, it was further discovered that spreading area and cell shape can profoundly affect traction forces (7), differentiation (8), growth (9), and apoptosis (10).

Accumulating evidence indicates that adhesive cells respond profoundly to the dimension of adhesive surfaces. Most conventional studies have been performed in 2D environments, on either charged plastic or glass surfaces. Adhesive cells under such conditions form prominent actin bundles (stress fibers), large wedge-shaped focal adhesions, and broad lamellipodia (11). In contrast, cells migrating along narrow lines form fewer interior stress fibers but strong peripheral actin bundles and small punctuate adhesion structures (12). Furthermore, centrosomes in cells on

1D substrate typically trail the nucleus (12), whereas centrosomes on 2D surfaces are typically located in front of the nucleus (12,13). Cells in 3D extracellular matrices (ECMs) are often stellar in shape and share many characteristics with cells in 1D (12,14), likely due to the fibrillar structure of many ECM proteins.

Physical cues must be sensed by cells with some form of physical interactions. Traction forces, myosin-II-dependent mechanical forces exerted by adhesive cells on the substrate (15,16), were believed to be the driving force for cell migration (17). However, there is increasing evidence to suggest that the role they play in sensing the physical environment and guiding cell migration (6) is at least equal in importance. The concentration of active traction forces near the leading edge, where protrusion and steering of cell migration take place, supports this view (15,17). Traction forces may be used for probing the stiffness of the substrate based on the deformability of the material upon mechanical stress. Cell shape and size can also be measured based on the amount of traction force required to maintain a mechanical equilibrium (7,18). A similar mechanism may be used to detect whether a cell is spreading over 2D surfaces or stretching along a 1D line.

Given the sensitivity of cell structures to substrate dimension, we hypothesized that migrating cells may be able to use dimension as a guidance cue. However, most studies of cell migration have focused on cells migrating in a homogeneous dimension, whereas any systematic investigation of dimension-mediated guidance must place cells on substrates with changing dimension. In this study, we created such an environment by micropatterning flexible polyacrylamide surfaces with alternating 1D lines and 2D rectangles of identical adhesiveness, which allowed us both to detect dimensional preference during cell migration and to measure the underlying traction stress. We further investigate whether dimension sensing might be impaired in transformed cells.

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MATERIALS AND METHODS

Preparation of patterned polyacrylamide substrates

Patterned polyacrylamide hydrogels were prepared as described previously (7). A 0.1% solution of 50 Bloom gelatin was activated with 3.6 mg/mL sodium m-periodate (Sigma-Aldrich, St. Louis, MO) at room temperature for 30 min. A polydimethylsiloxane stamp was fabricated by standard soft lithography techniques and incubated with the activated gelatin solution for 45 min. Excess solution was blown away under a nitrogen stream and the stamp was brought into contact with a small glass coverslip for 5 min.

Polyacrylamide was prepared with a final concentration of 5% acrylamide (Bio-Rad, Hercules, CA), 0.1% bis-acrylamide (Bio-Rad), and a 1:2000 dilution of 0.2- μm fluorescent beads (Molecular Probes, Carlsbad, CA). Initiators ammonium persulfate (Sigma-Aldrich) and N,N,N',N' tetramethylethylenediamine (Bio-Rad) were added to the acrylamide solution after degassing, and a 30- μL drop was pipetted onto a large coverslip activated with Bind-Silane (GE Healthcare, Waukesha, WI). The small stamped coverslip carrying activated gelatin was placed pattern-side down onto the acrylamide drop. After polymerization was complete, the top coverslip was gently removed. Patterned polyacrylamide hydrogels were mounted into chamber dishes, sterilized under ultraviolet light for 30 min, and incubated in cell culture media for 1 h at 37°C. The final gel had an estimated Young's modulus of 5.8 kPa.

Cell culture and microscopy

NIH 3T3 cells and PAP2 cells (Dr. Ann Chambers, London Regional Cancer Program, Ontario, Canada) were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, CA) supplemented with 10% donor adult bovine serum (Thermo Scientific, Waltham, MA), 2 mM L-glutamine, 50 $\mu\text{g}/\text{mL}$ streptomycin, and 50 U/mL penicillin (Life Technologies). Cells were treated with 10 μM blebbistatin (Calbiochem, San Diego, CA) for 1 h to inhibit myosin contractility. In some experiments, cells were treated with 30 μM mitomycin (Calbiochem) for 2 h to inhibit mitosis.

Phase-contrast images of migrating cells were collected with a Nikon Eclipse Ti microscope using a 10 \times PlanFluor air objective. Images were collected every 10 min for a period of 20 h. To avoid the influence that neighboring cells might have on migration, only single cells were counted. For quantification of migration parameters, phase-contrast images were collected using a 20 \times PlanFluor air objective every 2 min for a period of 6 h. Persistence is given as a ratio of net migration distance divided by the total pathlength.

Cells seeded on patterned substrates were fixed in 4% formaldehyde and stained with phalloidin (Molecular Probes, Eugene, OR), and antibodies

against vinculin (Santa Cruz Biotechnology, Santa Cruz, CA) or Ser¹⁹-phosphorylated myosin regulatory light chain (MRLC, Cell Signaling Technology, Danvers, MA). Fluorescence images were collected using a 100 \times oil immersion lens. Focal adhesion size was quantified by thresholding each image and creating a binary mask in ImageJ (National Institutes of Health, Bethesda, MD). Fluorescence intensity was measured by subtracting the average background intensity and summing the total intensity over each cell using custom software.

Traction force microscopy

Phase-contrast images of single cells adhered to the pattern were collected with a Nikon Eclipse Ti microscope using a 40 \times PlanFluor air objective. Fluorescent images of the embedded beads near the surface of the hydrogel were taken before and after cells were removed with 0.05% Trypsin-EDTA (Life Technologies) to remove traction forces. Cell outlines were manually drawn and bead displacement fields computed using custom software. Traction stress maps were computed using the LIBTRC package (Dr. Micah Dembo, Boston University, Boston, MA (15)).

Statistical analysis

The mean \pm SE was determined, and unpaired two-tailed *t*-tests were performed using the Analysis ToolPak in Microsoft Excel. To determine the significance of cell distribution in 2D versus 1D, the chi-square statistic was calculated and the corresponding *p*-value was obtained in Microsoft Excel. The number of cells observed for each experiment is indicated in the figure captions.

RESULTS

Fibroblast preferential localization in 2D areas over 1D lines

To test the hypothesis that the dimension of adhesive environment is able to guide cell migration, we designed micropatterned substrates such that migrating cells encounter alternating 1D and 2D environments. We define 1D as a strip sufficiently narrow to confine the trajectory of the nucleus along a straight line. Our pattern consisted of 50 \times 100- μm rectangular 2D regions connected by 1D lines 10 μm in width and 400 μm in length (Fig. 1 A). Substrates

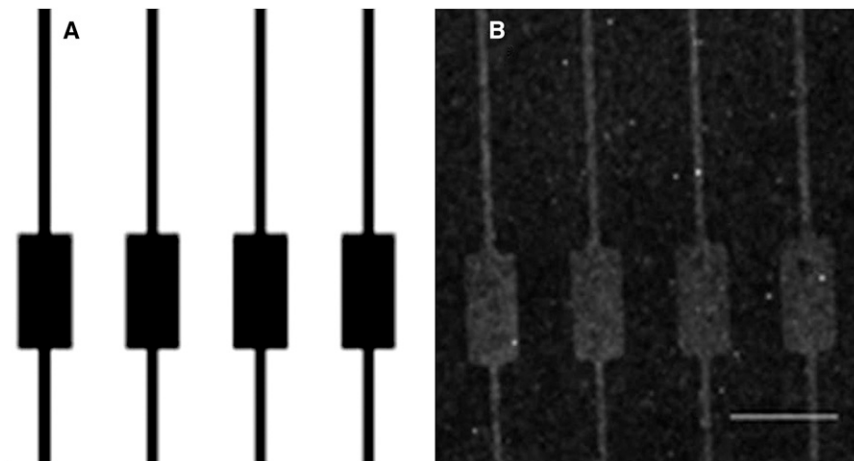


FIGURE 1 Micropattern with alternating 1D lines and 2D rectangles. The surface of polyacrylamide hydrogels is conjugated with gelatin in a defined micropattern (A), which is easily detected due to the concentration of fluorescent beads (B). Scale bar, 100 μm .

were generated by micropatterning gelatin on the surface of nonadhesive polyacrylamide sheets. Inadvertently we found that fluorescent polystyrene beads became more concentrated in areas conjugated with gelatin than in areas without gelatin, thereby allowing easy detection of the micropattern (Fig. 1 B).

NIH 3T3 cells were allowed to adhere on the micropatterned substrate, and their migration was recorded with time-lapse phase contrast microscopy. Preference between 1D and 2D regions was determined by counting the number of cells that were able to move across the dimension border versus those switching directions. We found that 100% of the cells entered 2D rectangular areas as they approached from a 1D line. These cells migrated persistently along the original direction (Table S1 in the Supporting Material) until the frontal process exited the 2D region and entered the 1D line on the opposite side. The majority of these cells

(63%, $N = 62$) then reversed the direction of migration and broke the persistence (Fig. 2 A and Movie S1). The response consequently caused cells to localize preferentially in 2D areas over time, such that the percentage of cells in 2D areas increased from 38% ($N = 684$) upon initial adhesion to 66% ($N = 685$) after 24 h of incubation (Fig. 2 C). This localization is not affected by cell proliferation, as similar results were obtained with cells maintained without or with mitomycin to inhibit mitosis (Fig. S1).

Involvement of myosin II and traction forces in dimension sensing

Due to the implication of myosin-II-dependent traction forces in sensing various physical cues, we hypothesized that the response to substrate dimension is dependent on traction forces. If true, a decrease in traction forces should reduce

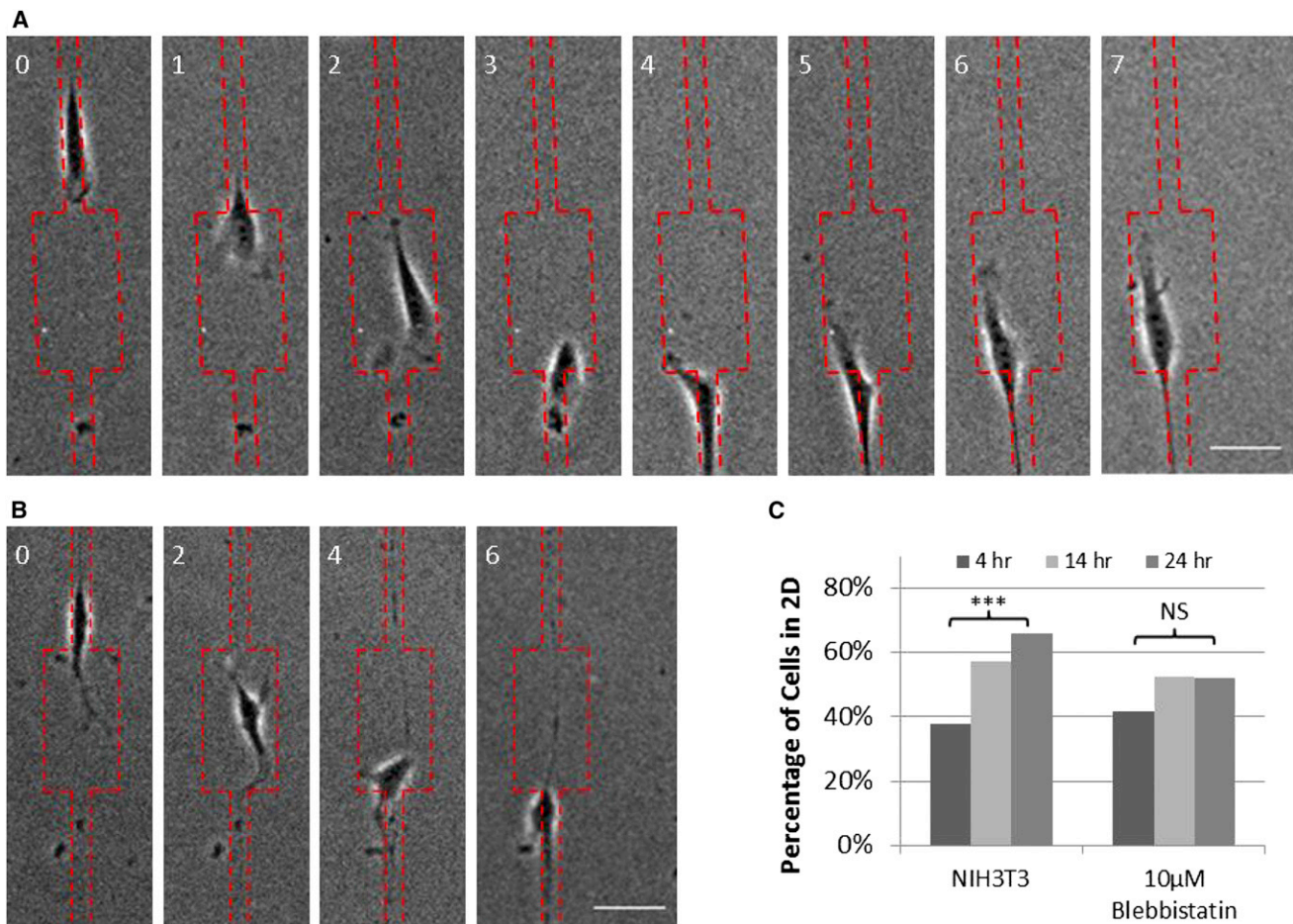


FIGURE 2 Different responses to the 1D-2D interface between normal and blebbistatin-treated cells. (A) An NIH 3T3 cell migrating along a 1D line enters a 2D area, then moves deeply into the 1D exit on the opposite side of the rectangle before turning around. (B) In contrast, a cell treated with 10 μM blebbistatin for 30 min enters the 2D area from a 1D line and exits through the 1D line on the opposite side. Red dotted lines indicate the borders of micropatterning. Numbers indicate time in hours. Scale bar, 50 μm . (C, left) As a result of their preferential localization on 2D areas, an increasing percentage of NIH 3T3 cells becomes localized on 2D areas over a period of 24 h after seeding. $N = 684, 701,$ and 685 cells at 3, 14, and 24 h, respectively (chi-square test, $* p < 0.0001$). (C, right) Cells treated with blebbistatin to inhibit myosin II show no significant accumulation on 2D areas. $N = 226, 202,$ and 184 blebbistatin-treated cells at 3, 14, and 24 h, respectively. The experiment was performed with cells treated with mitomycin.

the cell's ability to sense dimension. To test this hypothesis, we treated cells on micropatterned substrates with a potent inhibitor of myosin II ATPase, blebbistatin (19,20). Cells treated with 10 μ M blebbistatin maintained both their motility and persistence along 1D lines (12,21), as well as their ability to enter from 1D into 2D regions. However, the majority of cells (72%, $N = 36$) continued with the migration and exited into 1D lines at the opposite end (Fig. 2 B and Movie S2). Consistent with this finding, myosin-II-inhibited cells failed to show preferred localization on 2D areas after 24 h of incubation (Fig. 2 C), in contrast to control cells. The percentage of cells in 2D areas showed an insignificant change from 42% ($N = 226$) initially to 49% ($N = 184$).

A simple way for cells to detect the dimension border is to generate stronger traction forces on 2D surfaces than on 1D lines, which may bias both the direction of translocation and the strength of adhesive resistance due to inside-out signaling (22). To test this hypothesis, we quantified traction stress using traction-force microscopy (15). Comparisons were made based on the 95th percentile of traction stress,

i.e., the top 5% of traction stress exerted by each cell. This measurement is used to avoid the complication due to different cell areas. As shown in Fig. 3, A and B, cells migrating on 2D rectangles generated 44% higher 95th-percentile traction stress (1.37 ± 0.12 kPa) than cells along 1D lines (0.95 ± 0.08 kPa; $p = 0.008$). Time-lapse traction-force microscopy confirmed the increase in traction stress as cells cross the border from 1D to 2D (Fig. 3, C and D). In contrast, blebbistatin-treated cells showed not only a large decrease in magnitude, but also similar traction stresses on 2D and 1D substrates (0.48 ± 0.05 and 0.44 ± 0.05 kPa, respectively), consistent with the hypothesis that dimension sensing is driven by differential traction forces.

To test whether cells exhibited stronger adhesion on 2D than on 1D, we examined the morphology and total area of focal adhesions. As shown in Fig. 4 A, cells on 2D areas formed large, elongated focal adhesions throughout the cell body, whereas cells on 1D lines showed adhesions mainly at the leading edge. The number of focal adhesions and total area of focal adhesions were significantly higher on 2D

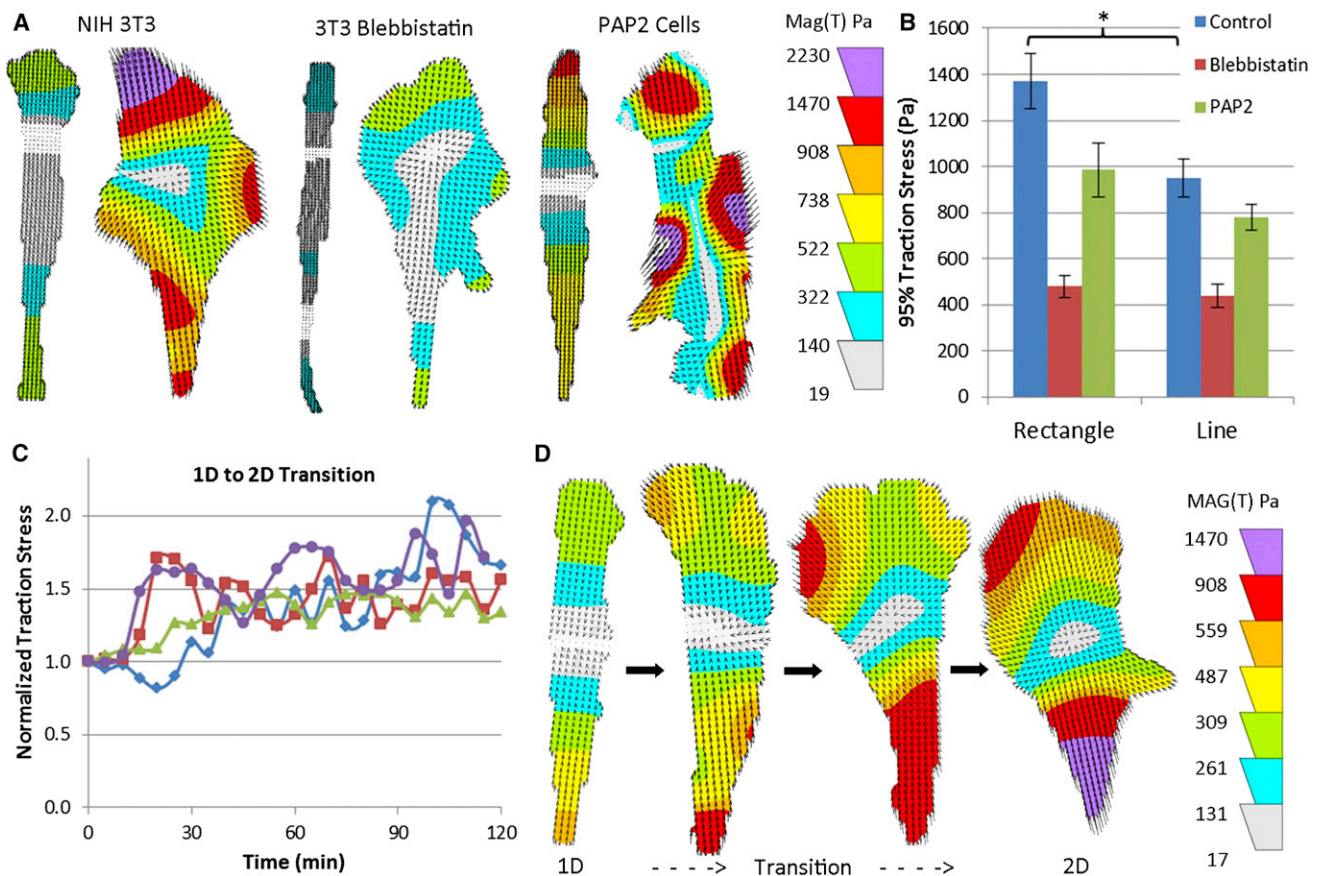


FIGURE 3 Traction stress measurements of cells migrating along 1D lines or on 2D rectangles. (A) The distribution of traction stress is shown as both vectors (small arrows) and heat maps (color-coded regions). (B) The bar graph shows the top 5% traction stress under different conditions. A significant difference between 2D and 1D is seen for control cells but not for blebbistatin-treated cells or PAP2 cells. $N = 18, 14,$ and 18 for control, blebbistatin, and PAP2 cells, respectively, on 2D rectangles. $N = 19, 15,$ and 19 for the corresponding measurements along 1D lines. Error bars represent the mean \pm SE (t -test, $*p = 0.008$). (C and D) Normalized traction stress of four cells (the time immediately before the cell reaches the 1D-2D interface is set as 0, and the corresponding traction stress is set as 1) and traction-stress heat maps (D) show an increase as cells migrate from 1D to 2D.

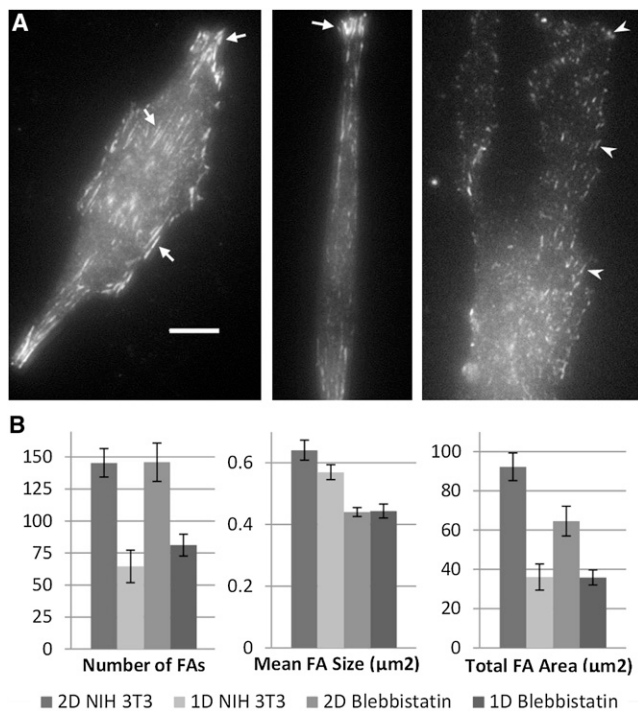


FIGURE 4 Size and number of focal adhesions along 1D lines and in 2D regions. (A) Immunofluorescence images of vinculin for NIH 3T3 cells show a larger number and/or size of focal adhesions in a 2D region than along a 1D line and after treatment with 10 μM blebbistatin. Arrows indicate elongated focal adhesions; arrowheads show small punctate adhesions in the cell treated with blebbistatin. Scale bar, 10 μm . (B) Bar graphs show average number of focal adhesions, focal adhesion size, and total focal adhesion area. Error bars represent the mean \pm SE.

than on 1D, and the average focal adhesion size was 14% larger in 2D under the present condition (Fig. 4 B). Staining with fluorescent phalloidin indicated that cells spread on 2D regions form thick stress fibers across the cell body, whereas cells on 1D lines show a strong actin cortex along the cell border but few stress fibers (Fig. 5 A).

As myosin II activities are regulated by the phosphorylation of its regulatory light chain at Ser¹⁹ and Thr¹⁸ (23), we stained cells on 1D and 2D with antibodies specific for MRLC monophosphorylated at Ser¹⁹. Phosphorylated myosin II generally colocalizes along the actin stress fibers. Interestingly, cells in 2D regions showed a strong concentration of phosphomyosin along stress fibers over the nucleus, referred to previously as the nuclear cap (Fig. 5 A). Staining of cells on 1D showed both weaker staining along stress fibers and fewer stress fibers, and only 25% of cells showed nuclear caps. Quantification of fluorescence intensity shows significantly more phosphorylated MRLC in cells spread on 2D surfaces compared to cells on 1D lines (Fig. 5 B). Blebbistatin-treated cells showed few actin stress fibers and small punctate focal adhesions along the edges on both 1D and 2D substrates (Figs. 4 A and 5 A). These results suggest a mechanism that limits traction-force generation based on physical constraints of the substrate.

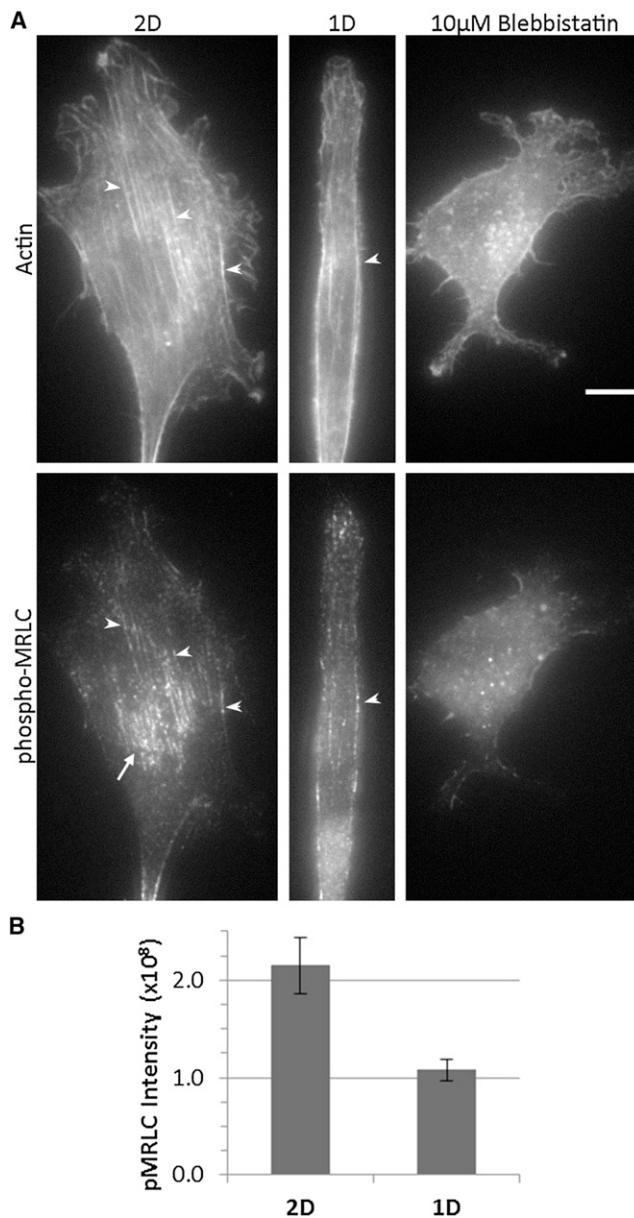


FIGURE 5 Stress fiber formation and myosin II activity differ between 1D and 2D. (A) Fluorescence images show the distribution of actin filaments and phosphorylated MRLC of NIH 3T3 cells in a 2D region, along a 1D line, and after treatment with 10 μM blebbistatin. Arrowheads indicate colocalization between phosphorylated MRLC and actin fibers. Arrow shows phosphorylated MRLC enrichment around the nucleus. Stress fibers are prominent in 2D regions, whereas cortical actin bundles are prominent in 1D. Treatment with blebbistatin causes disassembly of both forms of actin bundles. Scale bar, 10 μm . (B) Bar graph shows that MRLC is phosphorylated at a significantly higher level for cells spread on 2D surfaces compared to cells along 1D lines. Intensity is given in arbitrary units.

Defective dimension sensing for oncogene-transformed fibroblasts

As metastatic invasion may be caused by defects in migration guidance, we asked whether dimension sensing

might be affected in transformed cells, using *H-ras*-transformed mouse fibroblasts, the PAP2 line, as a model system (24). The NIH 3T3 cells used in this study are the parental cell line of PAP2 cells. Previous studies have shown that PAP2 cells generate disorganized traction stresses (25), such that the shape and migration of these cells are poorly coordinated with the direction of traction forces, whereas normal migrating cells showed a well-defined long axis, with strong traction forces concentrated along the anterior border of the axis (25). Furthermore, the growth and apoptosis of PAP2 cells were nonresponsive to substrate stiffness (26), in contrast to normal fibroblasts, suggesting that their mechanosensing may be defective.

Time-lapse recording indicated that, as in the case of 3T3 cells, PAP2 cells migrated persistently along 1D lines and rarely switched direction. However, much like blebbistatin-treated cells, *H-ras*-transformed cells are more likely to exit 2D areas into 1D lines than are normal 3T3 cells (Table 1 and Movie S3). This defect is also reflected in the lack of accumulation in 2D areas over time (Fig. 6 B). Moreover, a significant percentage of these cells reversed the polarity upon the initial entry from 1D into a 2D area (31%, $N = 39$) and exited along the line of entry (Fig. 6 A and Movie S4). This reversal of direction was rarely observed with NIH 3T3 cells with or without blebbistatin treatment.

To determine whether the defect of PAP2 cells in dimension sensing was related to abnormal generation of traction forces, we measured traction stress of PAP2 cells along 1D lines and on 2D areas. As shown in Fig. 3 B, traction stress decreased significantly compared to normal cells on 2D regions, but not along 1D lines. As a result, there was a smaller (27%) difference between the traction stress produced on 1D lines and that produced on 2D rectangles (0.78 ± 0.06 kPa vs. 0.99 ± 0.12 kPa, respectively; $p = 0.12$), compared to 3T3 cells. These data suggest that defects in the generation and/or regulation of traction forces may play an important role in the defect of dimension sensing for transformed cells, which may in turn contribute to their invasive behavior.

TABLE 1 Quantification of migration direction as cells approach 1D from 2D rectangles

	Stays in rectangle	Leaves rectangle	
Control	63% (39)	37% (23)	
Blebbistatin	28% (10)	72% (26)	**
PAP2	26% (10)	74% (29)	***

The 2D area was 50 μm wide and the 1D area was 10 μm wide. In contrast to control 3T3 fibroblasts, which prefer to localize in 2D rectangular areas, the majority of blebbistatin-treated cells and PAP2 cells readily leave 2D areas and migrate into 1D lines (chi-squared test with Yates correction, $**p = 0.0017$, $***p = 0.0006$). Only single cells that migrated into the 2D region were counted. Cells that remained in the rectangle for more than 6 h were counted as staying.

DISCUSSION

Elastic polyacrylamide hydrogels have been used extensively for testing cellular response to substrate rigidity and for measuring traction stress (6,27). A new method for high-resolution micropatterning of polyacrylamide surfaces further allowed the control of cell shape and migration and analyses of cellular responses to geometric parameters (7). In this study, we have applied these methods to test the sensitivity of cell migration to substrate dimension. Although true 1D lines rarely occur *in vivo*, they may be used as a simplified model for 3D migration because of the similarity in cell morphology, likely due to the fibrillar nature of the extracellular matrix. Recent work has investigated the effect of line width and substrate stiffness on cell migration (28). Here, we focus on cellular behavior at the border between 1D lines and 2D surfaces, by forcing NIH 3T3 fibroblasts to migrate between micropatterned alternating 1D and 2D environments. The striking morphological and structural differences, including lamellipodia, actin structures, and substrate adhesions, suggest that cells may show a preferential localization between 1D and 2D environments.

We found that when migrating cells encountered a transition from 1D to 2D, 100% of them entered the 2D area, which may be due in part to the strong persistence while migrating along 1D. In contrast, when cells encountered a transition from 2D to 1D, only a minority of them were able to continue into 1D lines. This difference cannot be explained by a difference in persistence, since the majority of these cells turned around only after a large portion of the cell had entered the 1D region where the persistence is higher than on 2D (Fig. 2 A and Table S1). Moreover, 3T3 cells rarely turned around when migrating across the length of 2D rectangles (Fig. 2 A). This active reversal of polarity supports the argument that the cells exhibit a real preference for 2D.

Two observations suggest that the preference for 2D over 1D is driven by differential traction forces. First, we found that cells generate stronger traction stresses when migrating on 2D surfaces than when moving along 1D lines. Although active traction forces at the front are always balanced by passive anchorage forces at the rear (15) (Fig. 3 A and D), these active forces are stronger when the frontal region is in a 2D region than when it is along a 1D line. The bias may then steer migration toward 2D. Second, the preference for 2D vanished when actomyosin contractility was pharmacologically blocked by blebbistatin, supporting the idea that the increase in traction forces on 2D is responsible for the preferential localization. Morphologically, cells treated with blebbistatin became elongated without a broad leading edge, resembling cells in 1D even after entering into 2D surfaces (4). Therefore, guidance by substrate dimension may be explained by the difference in size of the leading edge between 1D and 2D, which may differentially increase traction forces on 2D surfaces. Given the narrow leading edges for cells in most 3D ECMs (29), one could further

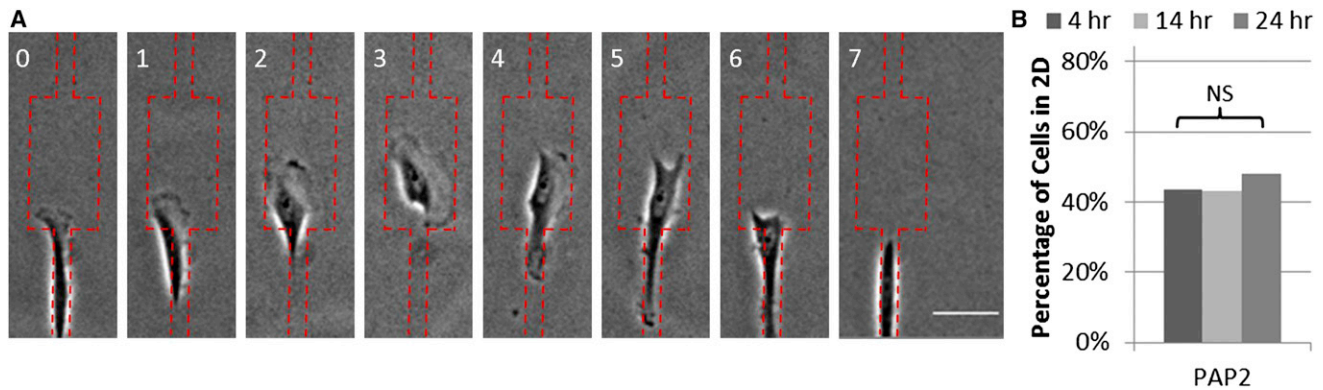


FIGURE 6 Lack of preference of *ras*-transformed NIH 3T3 fibroblasts (PAP2 cells) for localization in a 2D region. (A) A cell enters a 2D area from a 1D line but turns around to reenter the 1D line. Dotted lines indicate the border of the micropattern. Numbers indicate time in hours. Scale bar, 50 μm . (B) Consistent with a lack of dimensional preference, PAP2 cells show no significant accumulation on 2D areas over time. $N = 333, 351,$ and 386 cells at 3, 14, and 24 h, respectively.

predict that cells also prefer to localize on 2D surfaces when given a choice between 2D and 3D fibrous matrices.

The increase in traction stress on 2D is coupled to increases in focal adhesions and stress fibers. The maturation of focal adhesions, generation of traction forces, and assembly of stress fibers are likely coupled by positive feedback to reinforce each other (30,31). Thus the difference in traction-force generation may be attributed to the lower physical constraint for cell spreading on 2D than on 1D, which would allow the positive feedback to continue and brings traction forces to a higher level. Our results further confirm those of previous reports that focal adhesion size and traction forces are limited by geometric constraints of adhesion areas (7). Myosin II inhibition eliminates the effect of substrate dimension, as focal adhesions are unable to mature and grow in size regardless of the size of adhesion area. Furthermore, the intriguing localization of phosphorylated myosin II in nuclear caps of cells in 2D suggests strong contractility of stress fibers in the region above the nucleus, which may send forces along stress fibers to reach the associated focal adhesions at the cell anterior to mediate mechanosensing (32). In addition, it has been reported that microtubules may play a central role in regulating the actin cytoskeleton when cells are migrating in a confined channel (33). Observations from our group also showed that treatment with nocodazole inhibits cell migration along lines, yet blebbistatin and Y-27632 do not negatively affect migration speed (21). Thus, microtubule polymerization may provide at least some driving forces for 1D migration, whereas 2D migration requires primarily actin-myosin II contractility. These different mechanisms may generate different magnitudes of traction stress and cause the bias in cell localization.

As demonstrated by the preferential accumulation of cells on 2D surfaces over time, dimension sensitivity may be involved in concentrating cells at the destination during both physiological and pathological processes. Adhesive

ligands in multicellular organisms may be either concentrated along a network of fibers, creating a 1D-like environment, or distributed over a tissue surface, generating a 2D-like environment. The creation of such 2D surfaces may take place during embryonic development and wound healing, whereas 1D or 3D migration may occur during processes such as tumor metastasis (34). Other elements, such as soluble factors, immobile ligands, substrate rigidity, and mechanical forces, may play equally important roles and would allow multiple ways to regulate the destination of migration in a cell-type-specific manner.

We suspect that defects in dimension sensitivity may play a role in metastatic invasion, causing cancerous cells to leave their 2D home environment to invade into the surrounding fibrous connective tissue. Supporting this hypothesis, we found that *ras*-transformed 3T3 fibroblasts (PAP2) showed no dimension preference. Although PAP2 cells were as persistent as 3T3 cells when migrating along 1D lines (Table S1), many of them turned around when entering from 1D lines into 2D surfaces. This behavior was coupled to highly disorganized morphology and protrusive activities on 2D surfaces, with multiple protrusions seemingly competing against one another for the control of cell polarity (25). Traction stresses of PAP2 cells were also weaker and highly disorganized on 2D surfaces (25). Therefore, in addition to the smaller difference in traction stress between 2D and 1D, the unstable protrusions on 2D surfaces may prove less effective in guiding cell localization than the persistent migration imposed by 1D lines. A possible explanation of the defect at the molecular level may be the ability of *ras* to activate the PI3 kinase (35), which is in turn involved in the formation of lamellipodia from filopodia on 2D surfaces (36). In transformed cells, the formation of multiple competing protrusions may be attributed to a loss of regulatory control of *ras*.

Consistent with the idea that traction forces drive dimension sensitivity, PAP2 cells generated similar traction stress

in 1D and 2D environments. Interestingly, traction stress of PAP2 cells along 1D lines did not decrease significantly compared to nontransformed fibroblasts, supporting the idea that transformation may not affect the initial protrusion but instead may impair the subsequent expansion, stabilization, and force generation of lamellipodia on 2D surfaces. The lack of difference in traction stress between 1D and 2D may contribute to the defect in dimension sensing and the invasive behavior of transformed cells. Conversely, migration defects of transformation may be suppressed when cells are confined to a 1D environment.

Traction forces actively generated near the leading edge are ideally suited for guiding cell migration (15–17). The magnitude and pattern of traction forces, coupled to mechanical responses of the environment upon cellular probing, may allow cells to respond to both external parameters such as rigidity and internal parameters such as cell shape, size, and migrating state. Dimension sensing represents a novel addition to this collection of sensing mechanisms. Detailed knowledge of these force-dependent responses may facilitate not only the design of scaffolds for engineering artificial tissues, but also clinical interventions of diseases, such as cancer, that depend on cell migration.

CONCLUSION

To investigate the ability of cells to sense substrate dimension, we have microfabricated elastic polyacrylamide substrates with adhesive regions alternating between 1D lines and 2D areas. Migrating NIH 3T3 cells preferentially localize in 2D areas over 1D lines through a mechanism that involves the generation of myosin-II-dependent traction forces. Furthermore, this type of mechanosensing is defective in *H-ras*-transformed fibroblasts. Knowledge of how cells respond to changes in substrate topography is important for the design of materials for tissue regeneration and for strategies for cancer treatment.

SUPPORTING MATERIAL

One figure, one table, four movies and their legends are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(12\)05079-5](http://www.biophysj.org/biophysj/supplemental/S0006-3495(12)05079-5).

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