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Migration and shape of cells on different interfaces*

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Impacts of microenvironments on cell migration have been reported in various interaction modes. A rapid tumor metastasis occurs along topological interfaces *in vivo*, such as the interface between the blood vessels and nerves. In this work, we culture MDA-MB231 cells at dish-liquid, dish-hydrogel, and hydrogel-liquid interfaces, respectively, to study how these different interfaces influence cell dynamics and morphology. Our results show that the migration mode of cells changes from an amoeboid motion to a mesenchymal motion but their speed do not change obviously if the interface changes from hydrogel-liquid to dish-liquid. In contrast, the migration mode of cells at a dish-hydrogel interface maintains as a mesenchymal motion, whereas their speed increases significantly.

Keywords: extracellular matrix, cell migration, cell morphology, migration mode

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1. Introduction

Biological micro-environments within tissues and organs are complex *in vivo*, leading to various interaction modes between cells and extracellular micro-environment.[1,2] During tumor metastasis, cancer cells first break through the basement membrane and invade into the surrounding collagen, then cells intravasate into blood vessels or lymphatic system and transfer to distant organ site.[2–4] Meanwhile, the propensity of tumor cells migrating along anatomic structures, like nerves and external vascular surfaces, has also been recognized: a rapid invasion of brain melanoma cells along microvascular channels has been observed.[5,6] In all these cases, cells migrate along different types of interfaces in micro-environments, involving adhesion on flat two-dimensional (2D) surface and surrounded by three-dimensional (3D) matrix.

Cell migration in 3D tissue network is highly impacted by ECM properties, i.e., protein composition, cross-linking structure, and pore size.[4,7,8] When cells migrate within the surrounding ECM, cross-linking network imposes a confined space on cell bodies.[9] On the other hand, migration on 2D surface requires adhesion to the substrate, a lamellipod or bleb at the front of cell body, and myosin-actin contraction pulling cells, which is a barrier-free migration. Therefore, cells migrating in 3D and 2D microenvironment behave differently in morphology and cytoskeleton organization.[10–13]

Moreover, recent studies have shown that the migration pattern of cells in 3D changes with the different physical properties of ECM, i.e., cells-matrix adhesion reduction or metalloproteinase inhibition leads to cell migration mode transitions from mesenchymal motion to amoeboid motion due to the substrate stiffness.[14–17]

In this work, we separate cell microenvironments into three types based on the topological interfaces: dish-liquid interface, dish-hydrogel interface, and hydrogel-liquid interface. Migration mode tuning caused by these micro-environments is often accompanied with adjustment of cell polarity. Therefore, we study the changes of cell shape during cell movement in these three types of microenvironments, in order to find the correlation between cells dynamic and morphology. Our results show that cells will change their migration mode from mesenchymal motion to amoeboid motion if the microenvironment is from a 2D hard substrate to hydrogel surface, but cells speed does not change much during this environment change. In contrast, if cells are not on hydrogel surface but covered by hydrogel, they will maintain the motion mode as that on the 2D hard substrate, but increase the migration speed.

2. Materials and methods

2.1. Cell line and culture

Highly metastatic breast tumor cell line MDA-MB231-GFP (from H. Lee Moffitt Cancer Center, Tampa, FL, USA) was cultured in DMEM with 4.5 g/L glucose, L-glutamine and supplemented with 10% fetal bovine serum (10099-141, Life Technology) and 1% penicillin/streptomycin (30-002-CI, Corning). We took 0.25% trypsin-EDTA (25-053-CI, Corning) applied for 1–2 min for cells detaching. All the cells were

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incubated in incubator with 37 °C with 5% CO_2 and were passaged every 5–6 days for a maximum of 20 passages.

2.2. Sample preparation

Collagen I and Matrigel are two types of natural hydrogel used in the system. A 100 μL solution of 2 mg/ml collagen I was prepared with 58 μL 3.44 mg/mL collagen I (354249, BD), 10 μL Phosphate Buffered Saline (PBS, 46-013-CM, Corning), 1.3 μL 1N NaOH (Sigma) and 30.7 μL MQ water right before the test. Then the collagen solution was spread on the Petri dishes and then incubated in 37 °C for 30 min to form 2 mg/mL collagen I hydrogel. Then, 10.7 mg/mL Matrigel (356237, Corning) was mixed with proper amount of culture medium to form 8 mg/mL Matrigel hydrogel after 30 min crosslinking in 37 °C. Cells suspension (1×10^4 cells/mL) was loaded onto the Petri dishes substrate, collagen substrate, or Matrigel substrate, respectively, during preparing samples at the dish-liquid and hydrogel-liquid interfaces.

For samples at dish-hydrogel interface, cells were cultured on Petri dishes for 12 h for attachment. Then culture medium was removed and 40 μL hydrogel (2 mg/mL collagen or 8 mg/mL Matrigel) was covered on cells. The elastic moduli of Collagen and Matrigel used in this experiment are around 25 Pa and 75 Pa, respectively. After collagen or Matrigel gelling, culture medium was added to cover the cells again (Fig. 1(f)). Cells were cultured on a hard bottom substrate without hydrogel coating as blank control for the experiment.

2.3. Time-dependent imaging and statistical analysis

The fluorescent time-lapse imaging was performed on an inverted microscope (Nikon, Ti-E) supported by an on-stage incubation system for cell culture (Tokai Hit) to maintain culture conditions (37 °C, 5% CO_2) during imaging. Images were collected every 5 min for more than 8 h (Figs. 1(a)–1(e)).

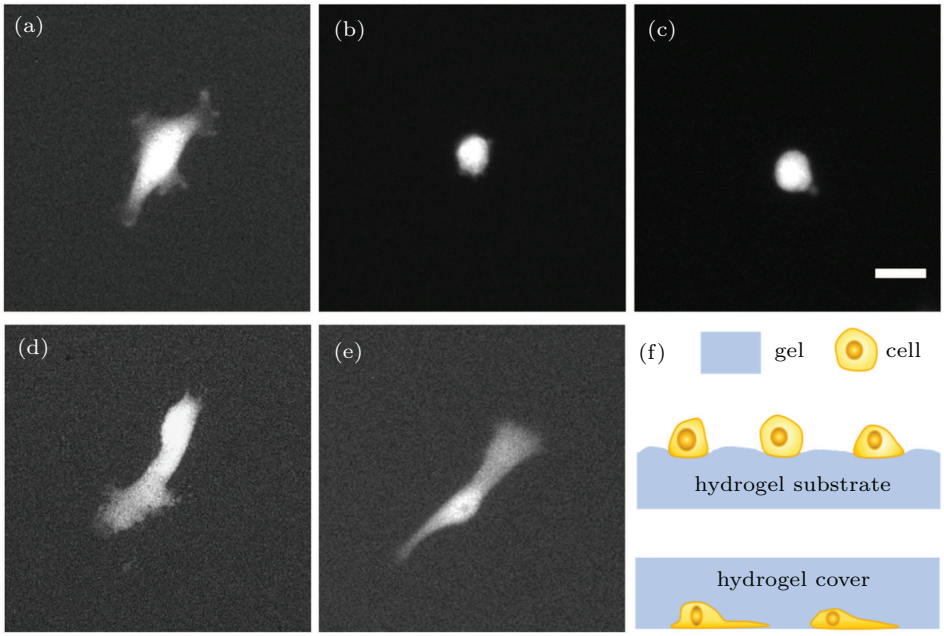


Fig. 1. Fluorescent images of MDA-MB231 cells under different conditions: (a) blank control; (b) collagen substrate; (c) Matrigel substrate; (d) collagen cover; (e) Matrigel cover; (f) schematic images of experiments design. The scale bar is 20 μm.

The gray-scale time-lapse images were further processed with ImageJ (NIH). Because there are multiple cells in one field of view, the raw images are cropped to track single cells. Then images were processed into binary images and used the "Analyze Particle" function to get the data of single cell positons, areas and perimeters at each time points.

Based on the cell positons during migration, the mean squared displacements (MSDs) can be calculated by

$$\mathrm{MSD}(n\cdot\Delta t) = \frac{1}{N-n+1}\sum_{i=0}^{N-n}\left[(x_{(i+n)\Delta t}-x_{i\Delta t})^2 + (y_{(i+n)\Delta t}-y_{i\Delta t})^2\right], \quad (1)$$

where Δt is the minimal time interval (between two frames), n represents the number of time intervals, N is the total number of time intervals per trajectory.

We define the shape factor S as the ratio between the perimeter P and the square root of the single cell area A,

$$S = P/\sqrt{A}. \quad (2)$$

When cell is in a round shape, S is equal to 3.5449, and the S value increases as cells become elongated.

Customized MATLAB (MathWorks) program was used to calculate the area and shape factor distribution. The "dfittool" tool box was applied to fit the data plotting.

3. Results

3.1. Effects of cell-ECM interaction on cell migration

We perform the quantitative MSD analysis of MDA-MB231 cells migration and average speed to investigate the dynamics of the cells on different types of the substrate interfaces. As shown in Fig. 2(a), on substrates of collagen I or Matrigel, MSDs of cell migration are similar. From Fick's laws, the MSD of a non-Brownian particle behavior in two dimensions is related to the cell diffusivity D,

$$\langle r^2 \rangle = 4Dt^{\alpha}. \tag{3}$$

If $\alpha = 1$, the diffusion is called Brownian motion; if $\alpha < 1$, the diffusion is related to the motion in confined space; and if $\alpha > 1$, the diffusion is called persistence random walk.[17,18] In Fig. 2(a), the slope α of MSD is around 1.5, implying that cells migrate more persistently. When we cover hydrogel on top of cells, α is still around 1.5, but cell diffusivity D increases (Fig. 2(b)). The average speed under different conditions also gives the similar result, which has higher speed for dish-hydrogel samples than that for dish-liquid samples. As shown in Fig. 2(c), MDA-MB231 cells do not have significant changes on average speed with different hydrogel substrates. However, when the hydrogel is on top of cells, cells migration has been speeded up.

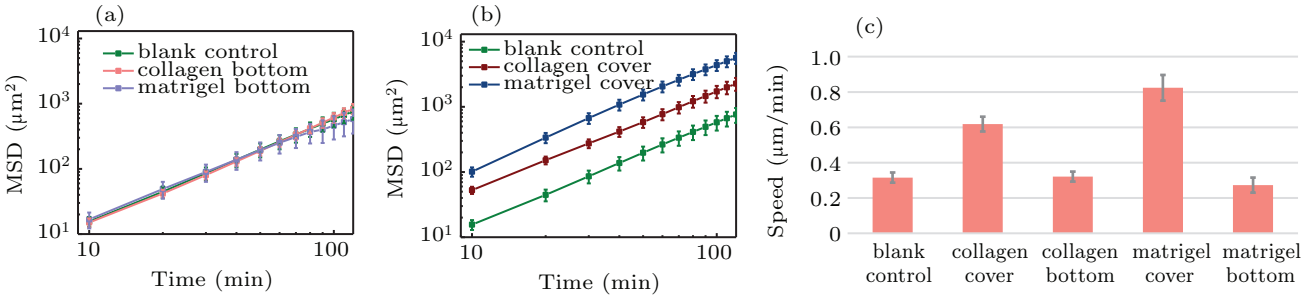


Fig. 2. (a) MSD of cells cultured on different substrates and (b) covered by various hydrogels. (c) Average cells speed under different conditions. The statistical analysis is calculated from about 20 cells with 50 time-points' tracking data.

3.2. Cell morphology changes with different cell-ECM interactions

Cell morphological changes usually are correlated to cell migration mode. We tracked cell area and shape factor variation during cells migration, which is normalized to the single cell's average value. The distributions of cell area and shape factor are fitted with a generalized extreme value distribution function using MATLAB. The average cell spreading area and shape factor is less for the cells on the hydrogel substrate compared to that on Petri dish, while there are no significant differences for cell morphology when cells are with or without hydrogel cover (Figs. 3(a3) and 3(b3)). Shape factors indicate the cell shape, which means that lower shape factor represents round cell shape and higher value represents spindle-shaped cells.

Cells frequently spread out and contraction leads to shape and area to change during the migration process. The distributions of normalized shape factor and area could give the frequency and amplitude of morphology changes. Compared to cells on the hard dish substrate, both area and shape factor distribution width are smaller for cells on hydrogels. MDA-MB231 cells spread blebby on hydrogel, so the magnitude of cell area changes is greater than the shape factor.

If hydrogel is covered on top of cells, the distribution widths of shape factor and area are larger than blank control group. Cells migrating at the hydrogel-dish interface also have higher velocity. Higher distribution width of cell area and shape factor of cells at hydrogel-dish interface indicate the large deformation of cells shape at this interface, which result in a higher speed during cell motion. The distribution width of cell area for cells under Matrigel cover is larger than that under collagen cover, which also matches to the speed differences.

3.3. Migration mode changes with different cell-ECM interactions

As shown in previous results, if cultured on collagen or Matrigel, cells shape becomes round, and cell spreading area goes down, but migration speed does not change dramatically. When cells are cultured under a hydrogel layer, there are no significant changes observed in cells' shape and area, but cells move faster than in blank control groups. The speed and morphology seem to have opposite trends when the substrates are changed. Therefore, we calculate the correlation between shape factor and velocity to see the statistical results.

When cells are cultured directly on dishes substrate, the slope of shape-speed correlation is about 0.12. However, if cells migrate on collagen and Matrigel substrate, the slope will increase to 0.27 and 0.44, respectively. If hydrogel covers on top of cells, the slopes of correlation are similar for collagen (0.14) and Matrigel (0.15), which means that cells speed up at the whole group level.

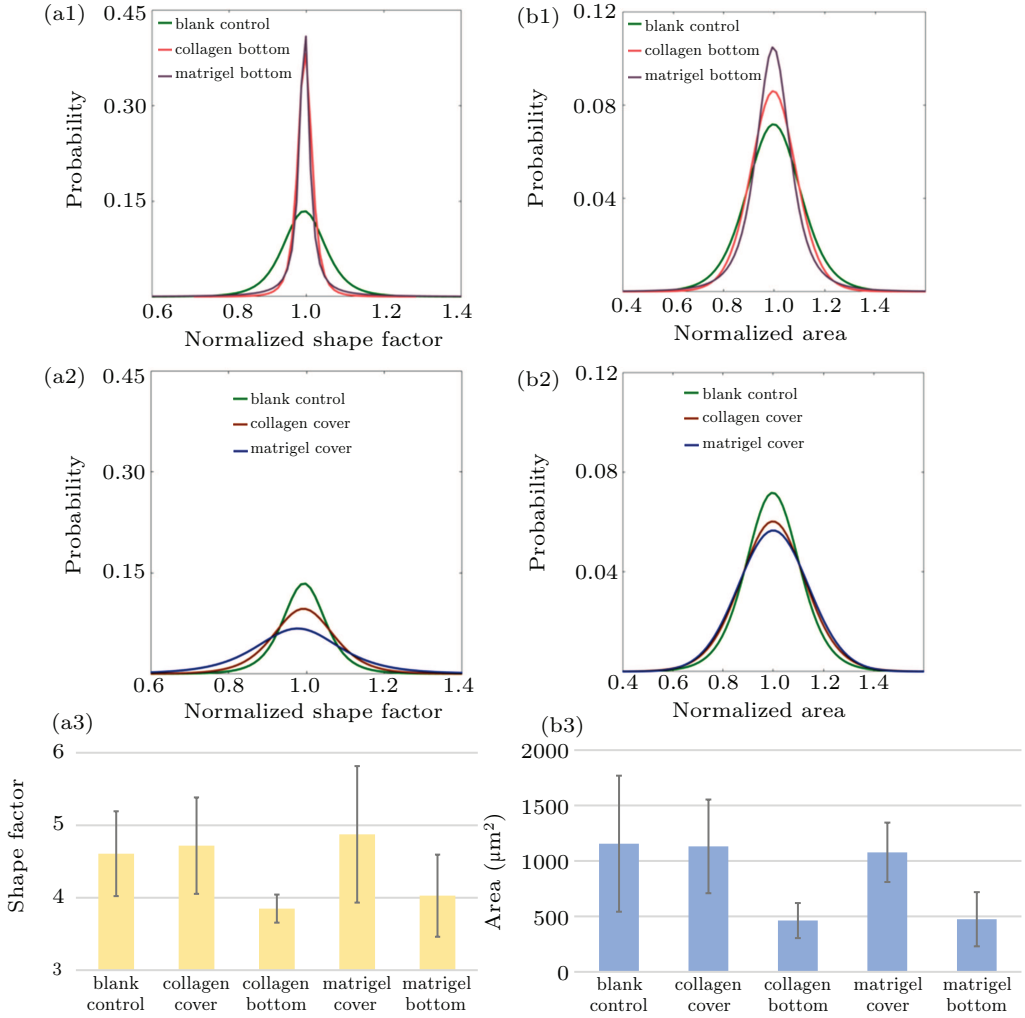


Fig. 3. Normalized cell shape factor (a1) and area (b1) distribution with different substrates. Normalized cell shape factor (a2) and area (b2) distribution with hydrogel cover. (a3) Average shape factor (a3) and area (b3) under all the conditions.

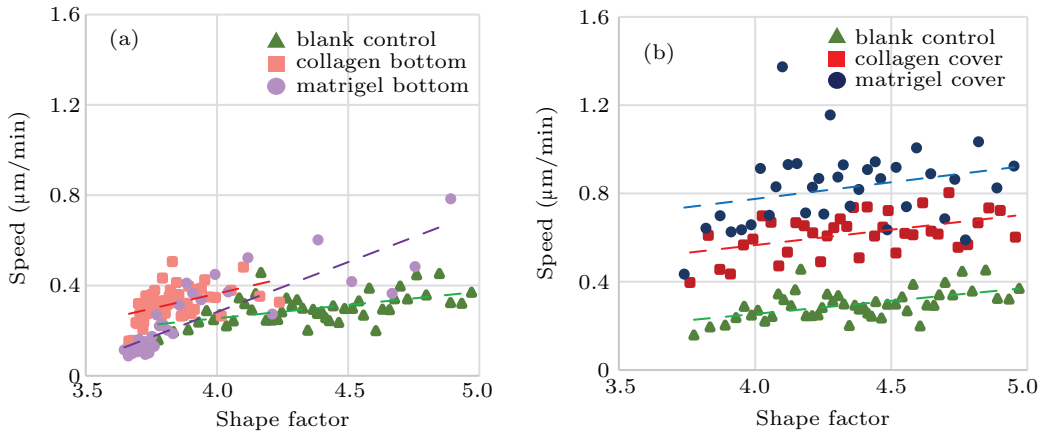


Fig. 4. Correlation between shape factor and cell speed with different substrate (a) and hydrogel cover (b). Each point means the average of 20 raw data points.

4. Discussion and conclusions

The modes of cell migration can be classified based on the morphology of migration patterns. When cells migrate on top of collagen or Matrigel, the cell shape is round with blebs and filopodia-lamellipodia, which is called the amoeboid migration mode. This kind of migration mode commonly refers to the cells lacking of mature focal adhesions. However, when the cells are covered with hydrogels, the cells become elongated and extend dynamic actin-rich dendrites to migrate as fibroblast. They have high levels of adhesion to the substrate and cytoskeletal contractility to develop a mesenchymal migration mode.[19]

Cell-ECM adhesions are also related to substrate type.

The substrate surface of commercial plastic Petri dishes has been pre-coated with specific proteins for enhancing cell adhesion and assisting tissue culture, so cell-dish adhesions are relatively tight. However, when cells are on softer hydrogel substrates, cell-ECM adhesion is weak, because a decrease in substrate stiffness would result in more dynamic Talin proteins and less stable adhesions, reflecting the relationship between substrate stiffness and mechanosensing network.[14,20] Therefore, MDA-MB231 cells change their migration mode from mesenchymal mode to amoeboid mode, as the substrate is changed from plastic bottom to soft hydrogel substrates. The slope of shape-speed correlation also increases when the substrate changes, which means that the slopes of correlation could serve as a reference to differentiate the cell migration mode.

When cells migrate under a hydrogel layer, migration mode and persistence do not change much as cells on the hydrogels, but cells speed increases obviously. This is due to the mechanical response of the cells to the stiffness of the surrounding environment. Myosin Ⅱ acts as the motor protein binds and maintains tension to actin fibers, which are coupled to the extracellular matrix by cell-matrix adhesion.[21] When the stiffness of the extracellular matrix is increased, the RhoA-ROCK-Myosin-Ⅱ pathway could enhance the cell actin-myosin contraction ability and promote maturation adhesions and cell directional movement.[7,11,22]

Also, Collagen and Matrigel are different on chemical components and microstructure. Collagen hydrogel is a kind of fiber material cross-linked with type I Collagen protein. Matrigel includes Lamin, Collagen Ⅳ, entactin/nidogen, and growth factors. Different from fibrillary Collagen I, type Ⅳ Collagen molecules organize into flat sheet-like network, and the pore size of 75% Matrigel is less than 2 μm. Integrins are one of the main cell-ECM adhesion receptors and many ECM and cell surface proteins bind to multiple integrin receptors with different α subunit and β subunit. For example, integrins $\alpha 2\beta 1$ and $\alpha 11\beta 1$ are binding to fibrillary Collagen, however, integrins $\alpha 1\beta 1$ and $\alpha 10\beta 1$ are binding to Collagen Ⅳ. Chemical properties of hydrogel and bio-chemical signals would also influence cell adhesion and mechanosensing molecular pathway, which are also responsible for cell migration.

In summary, our experimental investigations have revealed how interfaces in micro-environment influence cell migration mode, motility and morphology. From 2D hard dish to hydrogel surface, cells change their motion mode from mesenchymal to amoeboid due to the cell-ECM adhesion. When cells are covered by a hydrogel top-layer, the cell migration speed increases, probably because of their mechanical response to the substrate stiffness. Identifying cells' dynamics and morphology on different interfaces may provide further understandings to cellular behaviors in complex extracellular micro-environment.

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