Interplay among cell migration, shaping, and traction force on a matrix with cell-scale stiffness heterogeneity

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In living tissues where cells migrate, the spatial distribution of mechanical properties, especially matrix stiffness, is generally heterogeneous, with cell scales ranging from 10 to 1000 μm. Since cell migration in the body plays a critical role in morphogenesis, wound healing, and cancer metastasis, it is essential to understand the migratory dynamics on the matrix with cell-scale stiffness heterogeneity. In general, cell migration is driven by the extension and contraction of the cell body owing to the force from actin polymerization and myosin motors in the actomyosin cytoskeleton. When a cell is placed on a matrix with a simple stiffness gradient, directional migration called durotaxis emerges because of the asymmetric extension and contraction of the pseudopodia, which is accompanied by the asymmetric distribution of focal adhesions. Similarly, to determine cell migration on a matrix with cell-scale stiffness heterogeneity, the interaction between cell-scale stiffness heterogeneity and cellular responses, such as the dynamics of the cell-matrix adhesion site, intracellular prestress, and cell shape, should play a key role. In this review, we summarize systematic studies on the dynamics of cell migration, shaping, and traction force on a matrix with cell-scale stiffness heterogeneity using micro-elastically patterned hydrogels. We also outline the cell migration model based on cell-shaping dynamics that explains the general durotaxis induced by cell-scale stiffness heterogeneity. This review article is an extended version of the Japanese article, Dynamics of Cell Shaping and Migration on the Matrix with Cell-scale Stiffness-heterogeneity, published in SEIBUTSU BUTSURI Vol. 61, p.152-156 (2021).

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In response to the stiffness gradient of the extracellular matrix, cells undergo directional migration from soft to stiff regions, which should be an essential factor for various physiological and pathological processes. Since living tissues ubiquitously have cell-scale heterogeneity of matrix stiffness, the importance of cell-scale stiffness heterogeneity on directional migration called durotaxis should be clarified. Through an overview of systematic studies of cell migration on micro-elastically patterned hydrogels, we showed that the long-term migration trajectory is determined by the interactions among intracellular stress dynamics, cell shaping, and directional movement via the matrix with cell-scale stiffness heterogeneity.
Introduction

In our bodies, cells live in heterogeneous micromechanical environments. The stiffness of organisms—which is resistance to deformation in response to an applied force—varies by several orders of magnitude depending on the type of tissue, such as neurons (Young’s modulus E ~ 10^3 Pa), muscle (~10^4 Pa), and bone (~10^6 Pa) [1-3]. Even inside the same tissue, the microscopic spatial distribution of stiffness is heterogeneous, with spatial scales ranging from 10 to 1000 μm [4-7]. For example, in pituitary gland tissue, soft and stiff areas are heterogeneously distributed at the cell scale (2–20 μm), and the spatial gradient of Young’s modulus reaches ~10 kPa/μm [8]. In the bone marrow, sinusoids (~10^6 Pa) and trabecular bones (~10^6 Pa) coexist within a distance of 100 μm, and mesenchymal stromal cells are located around both perisinusoidal and peritrabecular areas [9]. Thus, the cells in our bodies must spontaneously migrate into a heterogeneous extracellular matrix (ECM), which often presents heterogeneity with a size scale of a few cells [10,11]. Since cell motility is deeply involved in various physiological and pathological processes, such as embryogenesis [12], immune response [13], and cancer metastasis [14], it is essential to reveal the migratory behavior of cells under cell-scale heterogeneity of matrix stiffness.

Cells can detect ECM stiffness and modulate cell–substrate adhesion, cell shape, and motility [15-18]. The actomyosin cytoskeleton generates contractile forces by myosin motors, and intracellular forces are transmitted to the ECM through cell-substrate adhesion sites called focal adhesions (FAs) [19]. On stiff ECM, the intracellular force can cause conformational changes in the proteins at adhesion sites, which recruit structural and signaling proteins to form and mature FAs. The maturation of FAs coincides with bundling and high organization of the actomyosin cytoskeleton, which forms actin stress fibers. As a result, cells spread on a stiff substrate with a polarized shape and generate large intracellular forces [20]. On the other hand, the soft substrate is largely deformed by the cellular forces applied to the substrate, whereas proteins in the adhesion sites are hardly distorted. Then, cells do not develop mature FAs and stress fibers, which results in a circular cell shape and small intracellular forces on the soft substrate [21]. The change in intracellular structures also modulates cell motility; cells have the largest speed at small or intermediate stiffness [20-23] because adequate adhesiveness is required for efficient movement [24]. Thus, interactions among intracellular forces, cell adhesion, and cell movement are key factors in understanding the effect of ECM stiffness on cell migration.

When soft and stiff substrates are joined with a straight boundary, adherent cells exhibit directional migration from the soft region to the stiff region, which is called durotaxis [25]. Although many studies have examined durotaxis on a broad stiffness gradient [26-29] and a single steep stiffness jump [25,30,31], cells can also sense a more general microscopic distribution of stiffness. To investigate the cell response to a matrix with a cell-scaled heterogeneous stiffness distribution, several types of elastically patterned substrates have been fabricated [32]: a) stripe-patterned polyacrylamide hydrogels fabricated by two-step polymerization [33]; b) photodegradable hydrogels [34]; c) photocurable styrenated gelatin (StG) gels [35] patterned by mask-free photolithography; and d) stepped micropillar arrays [36]. In this review, we summarize our studies on cell motility, shaping, adhesion, and traction forces at long time scales using micro-elastically patterned hydrogels [30,35,37] that enable us to systematically control the cell-scale heterogeneity of matrix stiffness. We also outline a cell migration model based on cell-shaping dynamics, which can reproduce general durotaxis driven by cell-scale heterogeneity of matrix stiffness [38,39].

Persistent Random Deformation Model on Homogeneous Substrate

As a control for cell migration on a matrix with stiffness heterogeneity, we first introduce experimental observations and a model of cell migration on hydrogels with homogeneous stiffness [38]. Adherent cells, such as fibroblasts and mesenchymal stem cells, migrate on the surface of the substrate by repeatedly elongating and contracting their pseudopodia through the growth of membrane protrusions driven by actin polymerization and contraction by actomyosin [40,41]. Consequently, on a timescale of hours to days, cells exhibit persistent random motion; they alternate between directed motion and random turning [42,43]. The persistent random walk model (PRW) has been widely used to describe persistent random motions [44-47]. However, the PRW model cannot explain motions with strong spatiotemporal correlations, such as back-and-forth, rotational, and zigzag motions, because it assumes Brownian motion. These ordered motions, which often appear on a time scale of several hours, correlate strongly with the dynamics of pseudopodia [48-51]. Therefore, to understand cell migration, it is necessary to consider fluctuations in cell shape, which are driven by the elongation and contraction of the pseudopodia.

A phase-field model has been proposed to explain the motions of keratocyte-like cells, which considers the coupling between the forces from motor proteins and the cell shape [52-54]. Phase-field models reproduce ordered motions, such as stick-slip motion [55] and bipedal motion [56] by considering the adhesion dynamics, traction force dynamics, and substrate elasticity and deformation. In active matter physics, ordered motions accompanied by deformation of the shape have also been investigated using a model of deformable self-propelled particles [57,58], which was applied to explain the cell shape and migration [38,59,60]. Compared with the model of deformable self-propelled particles, the phase-field...
model can describe two- or three-dimensional intercellular dynamics. However, phase-field models consist of partial differential equations and require heavy numerical computations. Because the models of deformable self-propelled particles are expressed as ordinary differential equations, they can be used to quantitatively compare and fit experimental data [38,60]. Here, we outline the experimental observations of cell shape fluctuations and migration, the distribution of which was numerically fitted by the migration model based on deformable self-propelled particles.

To experimentally elucidate the relationship between shape fluctuation and cell movement, the migration dynamics of fibroblasts were investigated on the surface of photocurable styrenated gelatin (StG) gels with various stiffnesses [38]. The typical time series of the cell shape and centroid are shown in Fig. 1 (a). Here, Fourier mode analysis of the shape was used to quantify cell-shaping dynamics (Fig. 1 (b)). The distance $R(\theta, t)$ from the centroid to the rim was calculated as a function of the angle $\theta$, which was measured from the $x$ axis. The complex Fourier coefficient, $C_n(t)$, was then calculated using

$$R(\theta, t) = R_0 + \sum C_n(t)e^{i\theta}$$

(1)

where $R_0$ denotes the mean radius of the cell. In this case, $C_2$ represents the magnitude and direction of uniaxial elongation of the cell. $C_3$ represents the magnitude and direction of the triangular deformation. $C_{\alpha}$ denotes the complex conjugate of $C_n$. By analyzing the correlation between the Fourier mode of the cell shape and the velocity of the cell centroid, the following relationship was obtained (Fig. 1 (c)):

$$v_i = \beta_1 \hat{C}_2 C_3 - \beta_2 C_2 \hat{C}_3$$

(2)

where $v_i = v_x + iv_y$ is the complex velocity, and $\beta_1$ and $\beta_2$ are the real constants. This velocity-shape relationship was equivalent to that of an amoeboid swimmer in fluids [61] despite the different physical processes between migration on the substrate and swimming in the fluid.

By incorporating the amoeboid swimmer-like velocity equation (2) into the model equations for a deformable self-propelled particle [59,60], we formulated the cell migration driven by the extension and contraction of the cell body (persistent random deformation (PRD) model). In the PRD model, cell deformation $C_n$ was assumed to be induced by the intracellular force $F_n$ acting on the cell periphery, which is driven by actin filaments (Fig. 2 (a)).

$$\dot{C}_2 = -\kappa_2 C_2 - \alpha_{3} v_x C_3 + F_2,$$

$$\dot{C}_3 = -\kappa_3 C_3 - \alpha_{2} v_x C_2 + \beta_1 C_3 F_6 + F_3,$$

$$K_{\alpha} = \kappa_3 + \gamma_3 |C_3|^2.$$  

(3)
where \( \kappa_n, \alpha_n, \beta_n, \) and \( \gamma_n \) are the positive constants. The nonlinear functions of \( C_n \) and \( F_n \) were derived from the spatial argument (see details in Ref. [38]). Here, \( F_n \) is considered to fluctuate randomly with a persistent time \( T_f \):

\[
\dot{F}_n = (-F_n + \sigma_n \xi_n) / T_f
\]

where \( \xi_n \) and \( \sigma_n \) are the white Gaussian noise and magnitude of the random force, respectively. In the experiment, cells moved along the long axis of elongation of their bodies, indicating a strong coupling between \( v_1 \) and \( C_2 \). Since Eq. (2) is not sufficient to reproduce such a movement, a coupling term between \( v_1 \) and \( C_2 \) is added to Eq. (2):

\[ \frac{\partial F}{\partial x} = \frac{\partial F}{\partial y} = \frac{\partial F}{\partial z} = \frac{\partial F}{\partial \theta} = 0 \]

\[ \frac{\partial F}{\partial r} = \frac{\partial F}{\partial \varphi} = \frac{\partial F}{\partial \psi} = \frac{\partial F}{\partial \zeta} = \frac{\partial F}{\partial \kappa} = \frac{\partial F}{\partial \alpha} = \frac{\partial F}{\partial \beta} = \frac{\partial F}{\partial \gamma} = 0 \]

\[ \frac{\partial F}{\partial r} = \frac{\partial F}{\partial \varphi} = \frac{\partial F}{\partial \psi} = \frac{\partial F}{\partial \zeta} = \frac{\partial F}{\partial \kappa} = \frac{\partial F}{\partial \alpha} = \frac{\partial F}{\partial \beta} = \frac{\partial F}{\partial \gamma} = 0 \]

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Through the numerical calculations of Eqs. (3)–(5), the experimental data were fitted to determine all the coefficients in the equations (the detailed values of the fitting parameters are shown in [38]). The PRD model quantitatively reproduced the statistical properties of the velocity, shape, and trajectory of fibroblasts (Fig. 2(b)). In particular, the conventional persistent random walk model cannot explain the characteristic reciprocating motion of fibroblasts on gels (Fig. 2(c)). However, the PRD model can reproduce the reciprocating motion because of the time-reverse symmetry of the amoeboid swimmer-like velocity equation, Eq. (2). The reciprocal property of the trajectories calculated from the model can be confirmed from the probability distribution of the turning angle $\Delta \theta$ of the trajectory, as schematically shown in Fig. 2(d). A large peak at 180° in the probability distribution of $\Delta \theta$ was reproduced by the simulation results, which indicated frequent reciprocal motion. In addition, the probability distribution of the persistent length $D$ of the trajectory and the mean square displacement of the cell centroid were simultaneously fitted by the model (Figs. 2(e, f)). Because the PRD model also reproduced the migration of mesenchymal stem cells, the PRD model could be a general model of cell migration driven by extension and contraction of the pseudopodia.

**Development of Asymmetry of Durotactic Cells on the Single Stiffness Jump**

As shown in the previous section, cell migration on a homogeneous gel can be predicted from the dynamics of cell shape. The next question is how the cell-shaping dynamics relate to durotactic cell motion driven by a simple stiffness gradient. Pseudopodia dynamics are also important in durotaxis. A substrate with a monotonous stiffness gradient causes focal adhesions to distribute asymmetrically between the leading and rear edges of the cell [30]; this causes pseudopodia to extend and contract asymmetrically [62]. In the soft region, the maturation of focal adhesions is inhibited and the retraction of the pseudopodia is enhanced. In the stiff region, the focal adhesions mature and the pseudopodia can extend to stiff regions. As a result, cells migrate towards stiffer regions by extension and contraction of the body. During directional migration, durotactic cells have a polarized shape. Such polarized shapes have been observed in randomly crawling cells on homogeneous substrates [18]. For polarized cells on a homogeneous substrate [63,64] and adhesive islands [65], the sequential regulation of focal adhesion, cell shape, and migration has been reported, and the development of the focal adhesion orientation precedes the elongation of the cell body in a few hours [63]. As for cell polarization during durotaxis, the micro-elastically patterned gels can be a platform to investigate the sequential development of cell

![Figure 3](image)

**Figure 3**  (a) (upper figure) Schematic illustration of the durotaxis of the cell on the gel with single stiffness jump. (lower figure) Schematic illustration of the spatial elasticity variation. (b) Schematic illustration of symmetric and asymmetric shape described by the combination of the Fourier modes. (c) Time evolution of the magnitude of asymmetric shape $|C_2 - C_3|$. 0 h means the time when the cell front touches the elasticity boundary (Fig. 3(a) upper figure). (d) Dependence of $|C_2 - C_3|$ on the position of the cell centroid. Gray region indicates elasticity boundary (Fig. 3(a) lower figure). Modified figures from Ref. [66].
shape and focal adhesions.

To elucidate the detailed relationship between cell asymmetry and durotaxis, the time evolution of the asymmetric shape of durotactic fibroblasts was studied on gels with a single stiffness jump [66] (Fig. 3 (a)). In terms of the Fourier mode analysis of the cell shape, the front-rear asymmetry is expressed as a combination of uniaxial elongation (mode 2, C2) and triangular deformation (mode 3, C3); the magnitude of the asymmetric shape is represented by |C2 − C3| [59]. For pure elongated and triangular cells, the shapes were symmetric and had no polarity (Fig. 3 (b)). The shape became asymmetric when the cells contained both C2 and C3 (Fig. 3 (b)). The symmetric shape satisfied |C2 − C3| = 0, whereas the asymmetric shape gave |C2 − C3| ≠ 0. Figure 3 (c) shows the time evolution of |C2 − C3| of the durotactic cells that migrated from soft (~30 kPa) to stiff (~300 kPa) regions. When the cell moved from the soft region to regions with a stiffness gradient, |C2 − C3| gradually grew over a period of 2–3 h. Subsequently, |C2 − C3| reached its maximum value and started to decrease slowly. In addition, |C2 − C3| in the boundary region became significantly large compared to |C2 − C3| on the plain soft regions (Fig. 3 (d)). These results indicate that cell elongation and triangular deformation (extension of the lamellipodia) were enhanced in response to the stiffness gradient, and the magnitude of shape asymmetry grew on a scale of hours.

To reveal the sequential order of the responses of focal adhesions and cell shape to the stiffness gradient, the time evolution of focal adhesions in the anterior part of durotactic cells was investigated using fluorescence recovery after photobleaching (FRAP) [66]. The mobile fraction of paxillin and the half-time of fluorescence recovery were measured through live-cell imaging and FRAP analysis of Venus-paxillin in focal adhesions. The mobile fraction and recovery time of paxillin in the stiffness gradient region were significantly lower than in the stiff region. After the leading edge of the cell reached the stiff regions, the mobile fraction and recovery time of paxillin in the anterior part of the cell rapidly increased, and front-rear symmetry of focal adhesion dynamics was established within 30 min. Thus, the microscopic asymmetry of focal adhesions preceded the change in cell shape. Since the dynamics of focal adhesions relate to the generation of intracellular stress, the results suggest that the intracellular forces were regulated by the elasticity gradient, VE, which induced asymmetric cell shape and directional movement.

**Durotaxis on the Matrix with Cell-Scale Stiffness Heterogeneity**

We reviewed the dynamics of cell migration and shaping on gels with a single stiffness gradient. To understand cell migration in living tissues, it is essential to determine directional cell movement driven by a more general distribution of matrix stiffness. Previous studies on cell migration on elastically heterogeneous substrates revealed that the direction and magnitude of the induction of durotaxis depend on the stiffness of the soft region [31], magnitude of the stiffness gradient [30,67], and curvature of the boundary between soft and stiff regions [68]. These results imply that cells sense the general spatial modulation of stiffness and regulate directional cell movement. The soft and stiff domains in living tissues vary in size, ranging from single-cell size (10–100 μm) to multicellular size (100–1000 μm) [4–7]. Thus, as the next step, we focused on the dependency of cell shaping and migration on the cell-scale heterogeneity of the matrix stiffness [39].

Using the striped patterned gel with various sizes of patterning period, the cell migration was measured on the patterned gels with 100 μm unit width, where the cells almost always contacted the boundary between the soft and stiff regions (Figs. 4 (a, b)). Young’s moduli of the soft and stiff regions were set to ~20 and ~50 kPa, respectively. To emphasize the effect of the domain shapes and sizes, the stiffness gradient was chosen to be slightly smaller than the threshold value of the durotaxis on gels with a single stiffness jump [30,31]. The cell type dependence of durotaxis was also examined by comparing the migration dynamics of fibroblasts and MSCs, which have been used in the study of directional cell migration. As shown in Fig. 4 (a), the fibroblasts almost freely crossed the stiffness boundary and moved on both soft and stiff regions. In this case, durotaxis was not induced because the cells did not preferentially migrate toward stiff or soft regions. In contrast, most MSCs remained in the stiff region, and the cell shape elongated along the stiff stripes (Fig. 4 (b)). Localization in the stiff region indicated the induction of strong durotaxis even though the stiffness gradient (30 kPa / 50 μm) was smaller than the threshold for the induction of durotaxis (60 kPa / 50 μm) on the large square stiff domains [31]. Thus, the results showed that the threshold of the durotaxis significantly depended not only on the cell types [33] but also on the spatial distribution of the stiffness, such as stripe and square stiff domains.

Next, to systematically investigate the dependence of durotaxis on the spatial heterogeneity of the stiffness, cell migration dynamics were analyzed by changing the unit width of the stripe pattern. Fibroblast cells were almost equally distributed in both soft and stiff regions of narrow striped patterns with 100 μm unit width, whereas they were moderately localized in the wide stiff striped gels with 600 μm unit width (Fig. 4 (c)). MSCs were strongly localized in the stiff region of the 100 μm-striped gels, whereas they tended to stay at the boundary between the soft and stiff regions of the 600 μm-striped gels (Fig. 4 (d)). When the stripe-patterned gels were divided equally into soft, boundary, and stiff areas, the number of MSCs settling in the soft areas approached that in the stiff areas as the unit width of the stripes increased [39]. The results indicated that the durotaxis of fibroblasts was moderately strengthened on the wider and multicellular-sized stiff stripes, and the durotaxis of MSCs was significantly enhanced on the narrow and single cell-sized stiff stripes. The difference in the spatial heterogeneity of the stiffness also modulates the cell shape [39]. In the case of fibroblast cells, as
the unit width of the striped patterned gel became narrower, lamellipodia tended to expand more frequently. The fibroblasts then formed triangular shapes on the single cell-sized stripes, while they were weakly aligned against the stiff stripes. On the narrow and single-cell-sized stiff stripes, MSCs strongly elongated and aligned parallel to the stripes, but the formation of lamellipodia was slightly inhibited. As explained in the previous section, the cell shape responded to a single stiffness gradient (Figs. 3 (c) and (d)), which could affect the durotaxis. The results on the striped patterned gels also implied the importance of cell-shaping dynamics on general durotaxis.

To test whether the general behavior of durotaxis can be explained by the response of cell shape to stiffness gradients, the cellular response to stiffness gradients was introduced into the PRD model [38]. Before applying the PRD model, it was confirmed that the relation Eq. (2) for cells on striped patterned gels was maintained (data not shown). In the PRD model, the intracellular force \( F_n \) induced the extension and contraction of the cell periphery, which caused the time evolution of the cell shape \( C_n \). Because the experimental results indicated that the stiffness gradient \( \nabla E \) led to a change in the cell shape, the extrinsic force \( f_n \) was assumed to be generated in response to \( \nabla E \), which could induce the time evolution of \( C_n \) (Fig. 5 (a)). The phenomenological equations for \( f_2 \) and \( f_3 \) were introduced by the spatial symmetry argument.

\[
\dot{f}_2 = \frac{1}{T_e} \left( -f_2 + \mu_2 \nabla_x E C_3 \right)
\]

\[
\dot{f}_3 = \frac{1}{T_e} \left( -f_3 - \mu_3 \nabla_x E C_2 \right)
\]

where \( T_e \) is the time required for \( f_n \) to respond to a stiffness gradient. Real constants \( \mu_2 \) and \( \mu_3 \) represent the strength of the response to \( \nabla E \). For simplicity, the following variables were used to express the stiffness gradient:

Figure 4  (a, b) Migration and shaping dynamics of the fibroblasts (a) and MSC (b) on the striped gel with 100 µm unit width. Stiff regions are shown in gray. Color indicates the observation time (see color bar). Gray line represents the trajectory of the centroid. (c, d) Probability distribution of the cell position on the striped gel with various unit widths. Horizontal axis denotes the position \( x \) normalized by the unit width \( L \) of the stripe. Gray color indicates stiff region. (c) Fibroblasts (d) MSCs. Modified figures from Ref. [39].
\[ \nabla_i E = \partial_i E + i \partial_j E \]

\( \nabla_i E \) is a complex conjugate of \( \nabla_i E \). In the PRD model for durotactic cells, \( f_n \) was added to the right-hand side of Eq. (3). A positive \( \mu_2 \) enhances the uniaxial extension of the cell shape perpendicular to the stiffness gradient, while a positive \( \mu_3 \) induces extension of the lamellipodia toward the stiff region. Subsequently, positive \( \mu_2 \) and \( \mu_3 \) result in directional movement toward the stiff region, which quantitatively reproduces previous experiments [26,27] and numerical simulations [69,70] with a single stiffness gradient (see details in Ref. [39]).

Considering the response of the cell shape to the stiffness gradients, as mentioned above [39], the conditions \( \mu_2 \sim 0 \) and \( \mu_3 >> 0 \) simulate the migration dynamics of fibroblast cells, whereas \( \mu_2 >> 0 \) and \( \mu_3 < 0 \) model the migration dynamics of MSCs. To examine the PRD model with a stiffness gradient, numerical calculations of the model were performed in the parameter regions of \( \mu_2 \sim 0, \mu_3 >> 0, \) and \( \mu_2 >> 0, \mu_3 < 0 \) (Figs. 5 (b, c)). In the case of fibroblast cells (\( \mu_2 \sim 0, \mu_3 >> 0 \)), wider striped patterns enhanced the localization of the cells in stiff regions (Fig. 5 (d)). In contrast, MSCs (\( \mu_2 >> 0, \mu_3 < 0 \)) were strongly trapped on the narrow stiff stripes (Fig. 5 (e)). It also reproduced the cell alignment parallel to the stripes. In addition, the peak of the probability distribution of MSCs appeared around the stiffness boundary of wide stripes, which resembled the probability distribution of MSCs on softer stripe-patterned gels (supplementary information in [39]). Therefore, the responsiveness of the pseudopodia to stiffness gradients should determine the cell-type- and domain-size-dependent durotaxis on the matrix with stiffness heterogeneity.

These results show that durotaxis strongly depends on the cell-scale heterogeneity of stiffness distribution, even for modest changes in stiffness. Furthermore, the results indicate that the preferred stiff domain size for cell localization strongly depends on cell type. This suggests that different cell types spontaneously accumulate in different locations in the same tissue depending on the domain size of the soft and stiff regions.
In the previous sections, cell-shaping dynamics regulated cell migration on both the homogeneous substrate and the substrate with a heterogeneous stiffness distribution. Since cell shape is strongly related to the spatial distribution of intracellular stress [71-73], changes in cell shape should be accompanied by fluctuations in intracellular stress. Thus, intracellular stress dynamics (ISD) are also an essential factor in understanding the detailed mechanism of durotaxis on the matrix with cell-scale stiffness heterogeneity. Intracellular stress has been measured for cells on homogeneous substrates [25,74] and substrates with a single stiffness gradient [28,75,76], revealing that cells generally apply greater stress on a stiff substrate. In particular, a cell spanning a substrate with different stiffnesses exerts larger forces on the stiffer side, which causes the asymmetric distribution of the traction force under the cell [76]. As for the ISD, stable intracellular tensions with low temporal fluctuation are maintained for a single cell [77,78] and cell clusters [78,79] on the homogeneous substrate. However, until recently, little was known about the ISD on the matrix with cell-scale stiffness heterogeneity.

For both 2D and 3D experiments, traction force microscopy (TFM) has been widely used to measure cellular forces at cell adhesion interfaces, which reflect cellular contractility and intracellular stress [80-84]. On homogeneous gels, analytical solutions of the linear elastic theory, the Boussinesq solution, are often used to calculate the traction force [80,81]. However, when the elasticity of the substrate changes sharply under a cell, such an analytical solution is difficult.
to obtain. Thus, TFM based on a numerical calculation, such as the finite element method (FEM) [28,82], is applied to the cells on an elastically heterogeneous substrate. Recently, by combining the fabrication technique of micro-elastically patterned hydrogel with FEM-based TFM, long-term traction force dynamics on an elastically heterogeneous substrate were studied [85], which is explained in this section.

Long-term and whole-cell-scale dynamics of intracellular stress were investigated in MSCs migrating on a heterogeneous matrix [85]. To reveal long-term ISD accompanied by durotactic cell migration, two types of micro-elastically patterned hydrogels were used. One was the striped patterned gel, where the Young’s moduli of the soft and stiff regions were set to be equal to those in Fig. 4. On the striped patterned gels, cells showed domain-size-dependent durotaxis and settled in a certain region, as mentioned above (Fig. 4). Thus, the spatially averaged stiffness under the cell could be almost constant and fluctuate slightly around the mean value. Another patterned gel was a triangular patterned gel, where triangular stiff domains were periodically placed on the hexagonal lattices. When the stiff domain is convex toward the soft domain with a curvature of < 50 \( \mu \text{m} \), cells migrate from the stiff to the soft regions, which is called reverse durotaxis [68]. Thus, on the triangular patterned gels, forward and reverse durotaxis were induced at the side and corner of the stiff triangle, respectively. Such competing durotaxis leads to continuous cell movement among regions with various stiffness values. Subsequently, the stiffness under the cell fluctuated significantly.

To clarify how the migration on the gel with cell-scale stiffness heterogeneity interacted with the ISD, the dynamics of traction force on the micro-elastically patterned gels were analyzed using FEM-based TFM. Figures 6 (a) and (b) show representative time series of the cell position and traction field on the striped and triangular patterned gels, respectively. The black/white background represents the stiffness distribution of the gel. As the color darkened, the gel became stiffer. In the case of the striped patterned gel with 100 \( \mu \text{m} \) unit width, cells tended to exert large forces on the stiff regions and stayed around the center of the stiff domains (Fig. 6 (a)). Thus, a single cell-sized stiff region could enhance the strength of the traction force, which was sufficient to induce strong durotaxis, even if the stiffness gradient was insufficient to induce clear durotaxis on the wider stripes (Fig. 4 (d)). On the triangular patterned gel with a 254 \( \mu \text{m} \) unit width, large traction forces emerged around the soft region outside the corners of the stiff triangles (Fig. 6 (b)). Thus, the convex shape of the stiff domain significantly enhanced the magnitude of the traction force around the corner of the triangle. As a result, the cells were attracted toward the soft region outside the corners of the stiff triangles, which should account for reverse durotaxis. These results indicate that the characteristic traction force dynamics should be the cause of the emergence of differential responses of durotactic migration on the matrix with cell-sized stiffness heterogeneity.

The spatial distribution of the traction force suggests that the traction force regulated the durotactic motion. Long-term cell migration on a substrate with stiffness heterogeneity also affected the time evolution of the traction force. The cell trajectories in Figs. 6 (c)-(e) show the spatiotemporal fluctuation of traction stress as well as positional information of the cells; the colors of the trajectories represent the magnitude of the normalized mean traction stress at each point. Trajectories on the triangular patterned gels showed frequent shuttling between the soft and stiff regions (Fig. 6 (d)), which was significantly different from the trajectories on the striped patterned gels (Fig. 6 (c)). Reflecting the migration dynamics, the fluctuation of traction stress was moderate on the striped patterned gel with a 100 \( \mu \text{m} \) unit width (Fig. 6 (c)), while the traction stress on the triangular patterned gels largely fluctuated (Fig. 6 (d)). Compared to the cells on the patterned gels, the fluctuation of traction stress was small on the homogeneous gel [78] (Fig. 6 (e)). To characterize the magnitude of the temporal fluctuation of the traction stress, we calculated the standard deviation of the time series of the

**Figure 7** (a) Normalized standard deviation (SD) of the traction stress calculated from the time course of mean traction stress of a cell. Standard deviation of the traction stress was divided by the time-averaged traction stress. Soft: homogeneous soft gel. Stiff: homogeneous stiff gel. Stripe: striped patterned gel with 100 \( \mu \text{m} \) unit width. Triangle: Triangular patterned gel. (b) Normalized SD of the aspect ratio of the cell body. Standard deviations of the aspect ratio were divided by the time-averaged values. The Mann–Whitney U test was used to calculate the P value. * P < 0.05. ** P < 0.001. Soft: \( n = 39 \). Stiff: \( n = 28 \). Stripe: \( n = 32 \). Triangle: \( n = 46 \). For all data, MSCs were used.

Modified figures from Ref. [85].
traction stress divided by the average traction stress [86] (Fig. 7 (a)). The magnitude of the stress fluctuation on the triangular patterned gel was approximately two times larger than that on homogeneous gels. The fluctuations on the striped patterned gel were intermediate between those on the triangular patterned and homogeneous gels. The fluctuation of the traction stress should also be coupled with the fluctuation of cell shaping because the measured fluctuation of the cell aspect ratio was the largest on the triangular patterned gel (Fig. 7 (b)).

Eventually, on a matrix with cell-scale stiffness heterogeneity, traction stress dynamics exhibit long-term characteristic fluctuations depending on the microscopic patterns of stiffness. This coincides with the cell migration between soft and stiff regions. Thus, when the competing durotaxis was driven by the stiffness heterogeneity of the matrix, an extraordinarily large fluctuation in the ISD was induced, together with enhanced shape fluctuations.

**Conclusion**

This review provides an overview of systematic studies of cell migration on the matrix with cell-scale stiffness heterogeneity. Traction force microscopy revealed that durotactic motions should be controlled by the spatial distribution of traction stress at a certain moment [85] (Fig. 6), which should correlate with the distribution of focal adhesions [30] (FAs). Because the changes in the cell shape have a time retardation of a few hours to the response of the FAs to the stiffness gradient [66] (Fig. 3 (c)), the dynamics of FAs and intracellular stress should regulate the cell movement through the modulation of the cell shaping on the matrix with cell-scale stiffness heterogeneity [39] (Fig. 4). Meanwhile, the strength of the traction stress and cell elongation fluctuated on a time scale of several hours owing to the cell movement between soft and stiff regions (Fig. 7), which indicated that the migration could alternately modulate intracellular stress dynamics (ISD) and cell shaping through the variation of the matrix stiffness under the cell [85]. Thus, the long-term migration trajectory should be determined by the interactions between the ISD, cell shaping, and movement via the matrix with cell-scale stiffness heterogeneity.

Changes in cell shape and traction force coincide with variations in cytoskeletal tension, cytoskeletal remodeling, and nuclear deformation, which regulate various cellular functions [87-90]. Thus, the question is what kind of functional effects migrating cells on a matrix with a cell-scale stiffness-heterogeneity experience. Recently, we reported the functional modulation of MSCs that underwent large fluctuations in intracellular stress on a triangular patterned gel where dual-durotaxis was induced [91] (Figs. 6 (b, d)). On the triangular patterned gels, the amplified long-term fluctuations in ISD (Fig. 7) led to a significantly large magnitude of nuclear shape fluctuation and spatiotemporal chromatin fluctuation, while the amplified nuclear fluctuation diminished with the application of tension inhibitors. Under dual-durotaxis, mechanotransducers YAP and RUNX2 [92,93] delocalized inside and outside the nucleus between soft and stiff regions, which prevented the accumulation of the mechanosignals required for lineage specification [93,94]. We confirmed that dual-durotaxing MSCs cultured on triangular patterned gels for a week maintained their stemness. Furthermore, culturing on triangular patterned gels significantly suppressed the bias toward osteogenic differentiation in comparison to culturing on homogeneous stiff substrates. Gene expression analysis predicted that functional categories of cell survival, viability, movement, and migration were activated in dual-durotaxing MSCs, while functional categories of mortality, organismal death, apoptosis, and necrosis were inactivated. Thus, the enhanced fluctuation of intracellular stress due to migration on a matrix with cell-scale stiffness heterogeneity can activate various cell functions.

Our migration model (the PRD model) can reproduce general durotactic migration. Because the PRD model only considers the dynamics of the cell shape, the results obtained from the model do not depend on the internal structure of the cell as long as the shaping dynamics are identical. Then, by fitting the experimental data to the model, we can clarify how the physical parameters determine the coefficients in the model, such as motility parameters, relaxation time of shaping, magnitude of internal force, and magnitude of durotactic responses. Such parameterization by fitting can be used to classify migrating cells. However, it is difficult to clarify the physical meaning of the coefficients in the PRD models without fitting. In contrast, two-dimensional models, such as the phase-field model, directly describe the dynamics of cell shape, actin/myosin propulsion, adhesion, and traction force. Tactic motion on substrates with heterogeneous adhesion [55], heterogeneous elasticity [56], and cyclic stretches [95] has also been explained using phase-field models. Thus, the remaining problem is to derive our model from whole-cell scale migration models, for example, phase field models, by the reduction theory used in the localized pattern in reaction-diffusion systems [96] and self-propelled particles driven by the diffusion of surface-active molecules [97].

In the durotactic PRD model, Eqs.(6) and (7), only the minimum nonlinear coupling terms are considered to explain the domain-size-dependent durotaxis qualitatively. Thus, another issue is to elucidate how many coupling terms are needed to fully reproduce the durotactic responses in complex elasticity patterning, including triangular-patterned gels. For example, in the simulation, the peak of the probability distribution of the MSC position shifts slightly to the stiff region (Fig. 5 (e)). To reproduce the peak of the probability distribution at the boundary between the soft and stiff regions (Fig. 4 (d)), another term is required in Eq.(6).
The additional term $\mu_4 (\nabla E)^2$ causes uniaxial elongation of the cell body along stripes. Because cells migrate along the long axis of the body, they are trapped in the boundary region. In the model, the dynamics of the largest odd and even modes of the shape, $C_2$ and $C_3$, were considered because higher modes ($n > 3$) can be approximately described by the nonlinear terms of the lower modes, that is, $C_4 \propto C_2^2$, $C_5 \propto C_2^3$ [38]. However, because a higher mode of the shape ($n = 4$) can induce additional ordered motion, such as standing and traveling waves on the periphery of the self-propelled particle [98], the introduction of higher modes ($n > 3$) might also improve the accuracy of the RPD and durotactic PRD models. The number of possible coupling terms that include $C_n$ ($n > 3$) and $\nabla E$ can be large. Thus, it is challenging to develop a systematic methodology for determining the necessary and sufficient coupling terms to precisely fit experimental data. A possible approach is machine learning, which develops a method to determine the governing nonlinear equations from data [99-101].

In conclusion, cell-scale stiffness heterogeneity regulates not only cell migration but also various cell functions by shaping dynamics that correlate with intracellular stress fluctuations.

**Conflict of Interest**

The authors declare no competing interests.

**Author Contributions**

H. E and S. K wrote the paper.

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