

Geometrical constraints and physical crowding direct collective migration of fibroblasts

Man Chun Leong,¹ Sri Ram Krishna Vedula,² Chwee Teck Lim^{1,2,3,*} and Benoît Ladoux^{2,4,*}

¹NUS Graduate School for Integrative Sciences and Engineering; National University of Singapore; Singapore; ²Mechanobiology Institute; National University of Singapore; Singapore; ³Department of Bioengineering; National University of Singapore; Singapore; ⁴Laboratoire Matière et Systèmes Complexes; UMR 7057 CNRS; Université Paris Diderot; Paris, France

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Migrating cells constantly interact with their immediate microenvironment and neighbors. Although studies on single cell migration offer us insights into the molecular and biochemical signaling pathways, they cannot predict the influence of cell crowding and geometrical cues. Using microfabrication techniques, we examine the influence of cell density and geometrical constraints on migrating fibroblasts. Fibroblasts were allowed to migrate on fibronectin strips of different widths. Under such conditions, cells experience various physical guidance cues including boundary effect, confinement and contact inhibition from neighboring cells. Fibroblasts migrating along the edge of the fibronectin pattern exhibit spindle-like morphology, reminiscent of migrating cells within confined space and high cell density are associated with increased alignment and higher speed in migrating fibroblasts.

The migration of mesenchymal cells such as fibroblasts is a topic of significant interest because of its direct implications in numerous physiological and pathological condition in-vivo such as the re-epithelialization of wounds¹ and metastasis of tumor cells during cancer progression.² For example, cells can acquire more migratory mesenchymal phenotypes via Epithelial-Mesenchymal Transition (EMT) to escape the confinements imposed by the physical microenvironment.^{3,4}

The coordinated migration of closely associated or dense population of mesenchymal cells is recapitulated in many physiological events, but less studied than migration of individual mesenchymal cells. Although some argued that collective migration of cells pertains only to epithelial or epithelial-like cell sheet migration,⁵ the broad definition of collective migration encompasses both the hallmarks of epithelial-like cell migration and the movements of loosely or closely associated group of cells.⁶ Some examples includes neural crest cells migrating as streams during embryogenesis,⁶⁻⁸ migration of neurons in the rostral migratory stream⁹ and fibroblasts migration toward wound site in tissue repair. In the initial stages of tissue repair, fibroblasts proliferate rapidly, migrate together toward the injury site and accumulate there to lay down new collagen matrix.¹⁰ Moreover, fibroblasts have been reported to play a role in coordinating the movement of cancer cells during the collective invasion of squamous cell carcinoma through remodelling of ECM microenvironment.^{11,12}

Physical aspects of the ECM matrices such as stiffness, topography, ligand density and topography can alter migratory phenotypes and characteristics of mesenchymal cells.¹³⁻¹⁶ Earlier experiments performed on individual 3T3 fibroblasts migrating

on 1D, 2D and 3D ECM matrices suggest that the local cellular microenvironment has significant impacts at the cellular and molecular level, which in turn manifest in morphological changes and migratory phenotypes.^{13,17} In particular, fibroblast migration on 1D or dense 3D ECM environments renders them to adopt a highly elongated morphology and rely heavily on their acto-myosin contractility machinery for migration. In contrast, migration of fibroblast in the absence of confinement, as seen on 2D matrices, are characterized by slower and contractility-independent movements.¹³ However, most of the studies failed to appreciate that cell migration occurs not in isolation as single cells, but under the influence and presence of neighboring cells. The behavior of cells at higher densities (where collective effects can emerge from physical interactions between cells) cannot be extrapolated from the behavior of isolated migrating cells.^{18,19} It is now acknowledged that collective patterns can emerge in various systems including cells, bacteria and insects where each individual entity does not necessarily interact directly with one another.²⁰ In this regard, we probe the impact of ECM boundaries and cell crowding on the migratory characteristics of fibroblastic cells.²¹ We have recently shown that the collective migratory behavior of epithelial cells was strongly affected by the geometrical constraints of the environment.²¹ Here we present a study based on a similar experimental set-up on 3T3 fibroblasts that do not interact strongly with one another and preferentially migrate as individual cells.

This assay consist of a fibronectin pattern that includes strips of different widths ranging from ≈ 20 to 400 μm connected to a large rectangular seeding area. The different strips are initially

*Correspondence to: Chwee Teck Lim and Benoît Ladoux; Email: ctim@nus.edu.sg and benoit.ladoux@univ-paris-diderot.fr
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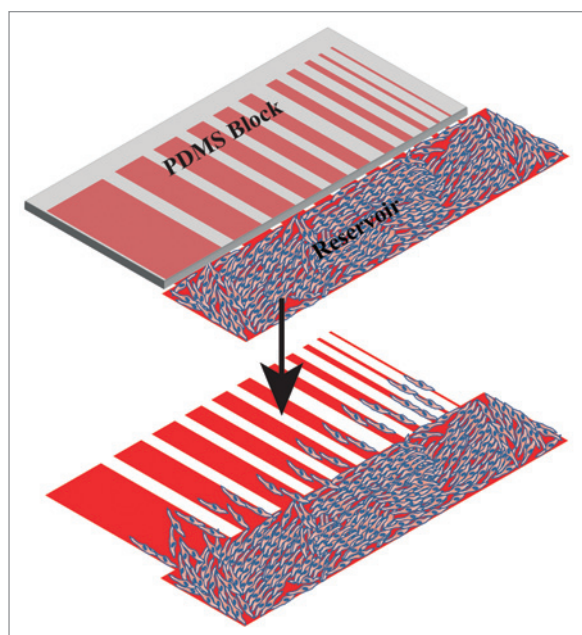


Figure 1. Schematic of the 3T3 cells presented with fibronectin line patterns with different lateral widths. The cells were allowed to migrate onto the lines pattern upon removal of the PDMS barrier.

covered with a polydimethylsiloxane block (PDMS) and cells are grown within the large rectangle. The PDMS block is later removed to allow cells migration on the fibronectin strips presenting different extent of lateral confinement (Fig. 1). A mixture of Cy-3 labeled fibronectin and unlabeled fibronectin are used and visual inspection of the Cy3 fluorescence intensity were performed after microcontact printing to ensure homogeneity in the fibronectin concentration. Systematic examination of migrating cells presented with wider 2D free space to narrow 1D ECM tracks was performed by analyzing the orientation, kinematics and morphology of migrating cells at different spatial locations. We observed different distinct behaviors arising from physical interaction of cells with one another and the geometry of their immediate ECM environment.

On the wider strips (400 μm and 200 μm), cells in the center region of the fibronectin strips were sparsely distributed, randomly oriented and exhibited a fan-shaped morphology that was typical of fibroblasts on a 2-D surface. In contrast, cells adjacent to the sides of the strips preferentially oriented themselves along the long axis of the pattern and took on a spindle-shaped morphology (Fig. 2A). We then asked whether this difference in cell morphology and orientation is accompanied by differences in migratory characteristics. Using Particle Image Velocimetry (PIV), we observed that average cell speed along the length of the strip decreases with increasing distance from the edges of the fibronectin strips (Fig. 2B). Concomitantly, the velocity vectors of cells nearer to the edges of the strips exhibited higher “orderliness” and alignment along the length of the fibronectin strip as reflected in the monotonous decrease in order parameter of the velocity vectors with increasing distance from the edge (Fig. 2C). In this case, the order parameter was taken to be absolute value of

the cosine of the angle that individual velocity vector makes with the length of the fibronectin strip.

Individual cell motility on the wider strips was further examined by tracking the locations of cells over time and computation of their mean squared displacement.²² We believed that the persistence time of migration of fibroblasts would differ depending on their spatial location on the wide fibronectin strip due to the different degree of confinement experienced. Cells with constrained cell shape are shown to respond more slowly to reorienting stimuli.²³ Moreover, the physical aspects of the ECM such as ECM alignment can alter the persistence of cell migration.²⁴ From the mean squared displacement data, we noticed that 3T3 fibroblasts which were within $\approx 50 \mu\text{m}$ from the lateral edges of the wider strips migrate in a highly ordered fashion, exhibiting more persistent and faster movements than those farther away. Average persistence time among cells within $\approx 50 \mu\text{m}$ from the lateral edge were approximately four times higher than that of cells farther away (Fig. 2D). Coincidentally, earlier experiments performed on NRK fibroblasts (that form weak intercellular adhesions) also show a correlation length of $\approx 50 \mu\text{m}$.²⁵ Together, we speculate that the migratory behavior of fibroblast on wide fibronectin strips exhibit two regimes. Although the cells are presented with a 2D topography, the morphology and migratory behavior of cells adjacent to the edge of the strip resembles that of cells on 1D surface. Cells which are located farther than $\approx 50 \mu\text{m}$ away from the edges are not able to sense and respond to the physical cue provided by the edge and conform to the typical fan-shaped morphology of fibroblast seen on 2D topography.

We then asked the question of how far the physical cue of an edge can be sensed by migrating fibroblasts. Migration of cells along fibronectin strips with a systematic reduction in lateral dimension (100 μm to 20 μm) was thus examined. Cells migrating on the narrow strips (20 μm , 30 μm) exhibited spindle-shaped morphology, typical of fibroblast on 1D topography and similar to cells adjacent to the lateral edges of the wide strips (Fig. 2A). This observation is in agreement with the results performed on 3T3 fibroblast which suggest that migratory phenotypes of cells in 1D is akin to that of 3D migration.²⁶ Cells migrating in the center on fibronectin strips of widths from ≈ 50 to 100 μm are also much more aligned along the long axis of the fibronectin strip (Fig. 2E). Average cell velocities were also higher than that in the wider strips. Interestingly, the phenomena of increased cell alignment and migratory velocity could also be seen in the wider strips with increasing cell density. Cells in the center of wide strips preferentially orient themselves along the length of the fibronectin strip as local cell density increases.

From a physical perspective, contact inhibition of cell movement and interaction of cells with the edge of the fibronectin strip can induce reduction in degree of freedom among cells closer to the boundary and in turn promote cell alignment. Complementing results from earlier studies which show that one-dimensional cell migration is akin to migration of cells in 3D matrices;²⁶ our results suggest that the interaction in dense clusters of cells on 2D surfaces could render cells to deploy similar migratory mechanisms as in 3D. We are also inclined to think that either one of the aforementioned physical factors alone is

sufficient to induce cell alignment and promote directed migration of fibroblast. These physical factors are ubiquitous in the physiological setting and can act in synergy to coordinate migration of fibroblastic cells.

Materials and Methods

The fibronectin patterns were prepared as previously described.²¹ 3T3 fibroblasts were used in the experiments and were maintained in DMEM (Hyclone) supplemented with 10% FBS (Hyclone) and 1% penicillin and streptomycin. Particle image velocimetry (PIV) was implemented in MATLAB to determine the velocity field, average velocity and order parameters were calculated from the velocity vectors. Centroid of at least 20 cells was tracked manually and was used to compute the mean square distance displacement. For the computation of persistence time, mean square displacement data were fitted to the equation:

$$\langle d^2 \rangle = 2S^2 T_p (t - T_p (1 - e^{-t/T_p}))$$

Where T_p and S denotes the persistence time and root-mean-square speed respectively.²⁴

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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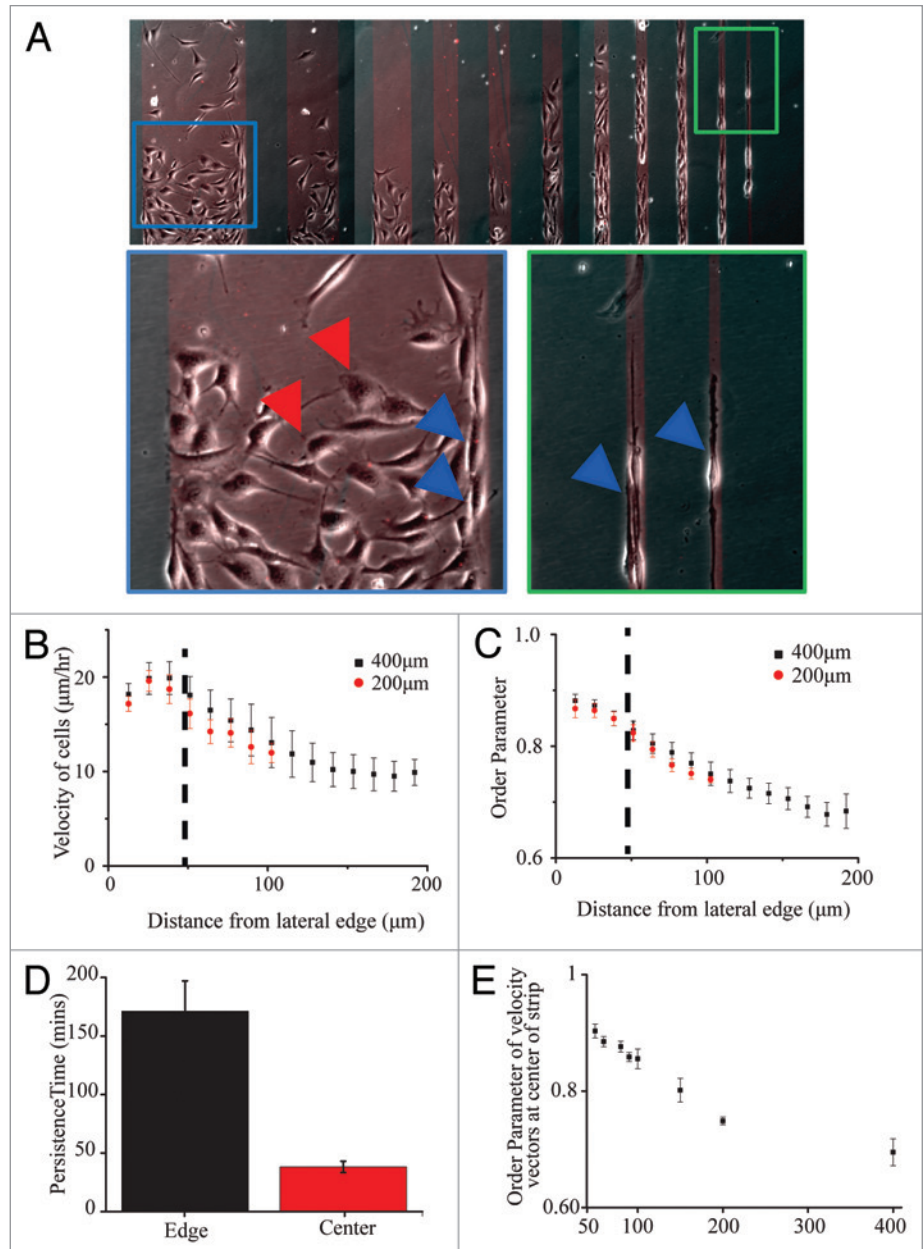


Figure 2. (A) 3T3 cells migrating on fibronectin strips with different lateral width. Magnified views of region denoted by blue and green box show cells on 400 μm wide (bottom left), 30 μm and 20 μm wide fibronectin patterns (bottom right) respectively. Red arrowheads indicate cells with fan-like morphology and black arrowheads indicate cells with spindle-shaped morphology. **(B)** Average speed of cells along strip length at different distance from the lateral edge and **(C)** average order parameter of cells. **(D)** Persistence time of movement of cells within 50 μm from the lateral boundary of wide fibronectin strips and cells in the center of the strips. **(E)** Average order parameter of cells in the center of strip decreases with increasing width of the fibronectin strip.

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