9

Controlling Cell Migration with Micropatterns

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1. Introduction

Long-distance and directional migration of cells is a critical step in development, regeneration, and wound healing. However, physical barriers such as connective tissues and other cells prevent cells from freely migrating towards their destination (Fig. 1a,b). Therefore, cells need to not only mechanically sense the surrounding geometry but they also need to integrate the mechanical information in their migration towards their destination. Technical limitations have meant that the relationship between the surrounding geometry and cell migration has not been well studied.

Recent advances in soft lithography techniques now allow various designs of micrometre-sized chambers to be easily fabricated on cell-culture vessels. By culturing cells in different micropatterns, the relationship between geometry and cell response has been studied (Fig. 1c,d). For example, spindle orientation, growth, differentiation, and migration have been shown to be related to micropattern shape. Recently, several groups, including ourselves, have reported that mammalian cells exhibit biased cell movement on asymmetrical periodic micropatterned surfaces. Although it is little wonder that cells migrate asymmetrically in asymmetrical micropatterns, the direction in which they move is not immediately obvious.

In this chapter, we describe biased cell movement in asymmetrical micropatterns. These studies offer new insights into the migration of cells in response to geometry of their surrounding environment, and we suggest strategies for designing artificial scaffolds that direct cell migration.

1.1 Biological significance of cell migration

1.1.1 Cell movement: A basic characteristic of life

Movement is a basic characteristic of cells (both unicellular organisms and the various cells of multicellular organisms) that is almost as important as self-renewal (Bray 2001). Some types of cells are extremely motile, while others lack strong motility and are capable of no more than passive movements caused by surrounding forces. Motile cells may sometimes change direction of their own accord in response to changes in their internal state (Oosawa 2001; Nakaoka et al. 2009), but normally they change direction in response to external stimuli, such as chemicals (chemotaxis) and light (phototaxis). For unicellular organisms, the ability to migrate to an environment suited to survival and proliferation is a matter of life and death. Bacteria search for an environment suited to survival by swimming. Social amoebae usually migrate independently of each other, but if the environment deteriorates, they gather to form fruiting bodies (Goldbeter 1996; Gregor et al. 2010). The fact that a great many of the cells of multicellular organisms (particularly animal cells) are capable of migration is also important. In the body plan of multicellular organisms, cells need to be able to do more than just proliferate and differentiate. In the process of development, cells need to migrate to the correct position so that they can adopt their proper shape and properly function (Keller et al. 2008). Cells also need to migrate *en masse* to specific locations to assist in the healing of wounds, to perform immune system functions, and to conduct other aspects of body maintenance (Friedl et al. 2004; Schneider et al. 2010). This suggests that motility is one of the universal characteristics of cells that enable the survival of life forms.

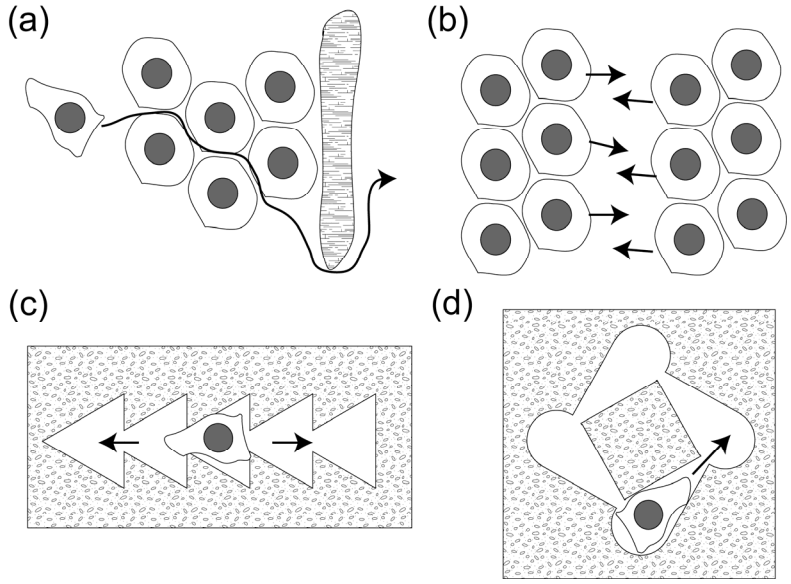

Fig. 1. Schematic illustration of cell migration in a multicellular body (a) and in wound healing (b). *In vitro* cell migration observed in asymmetric micropatterns connected in a linear (c) and circular manner (d).

1.1.2 Migration mechanisms

The way cells move differs markedly according to whether they are non-adherent or adherent cells (Eisenbach et al. 2004). Many non-adherent cells propel themselves using cilia or flagella. The structure of motor-protein complexes, energy balances, and protein response networks related to ciliary and flagellar locomotion have been analysed, and these locomotion mechanisms are becoming increasingly well understood. However, there is much that is still unknown about the movement of adherent cells because they lack specific means of movement such as cilia and flagella. This makes it difficult to clearly separate the different aspects of cell movement, such as deformation, migration, and division.

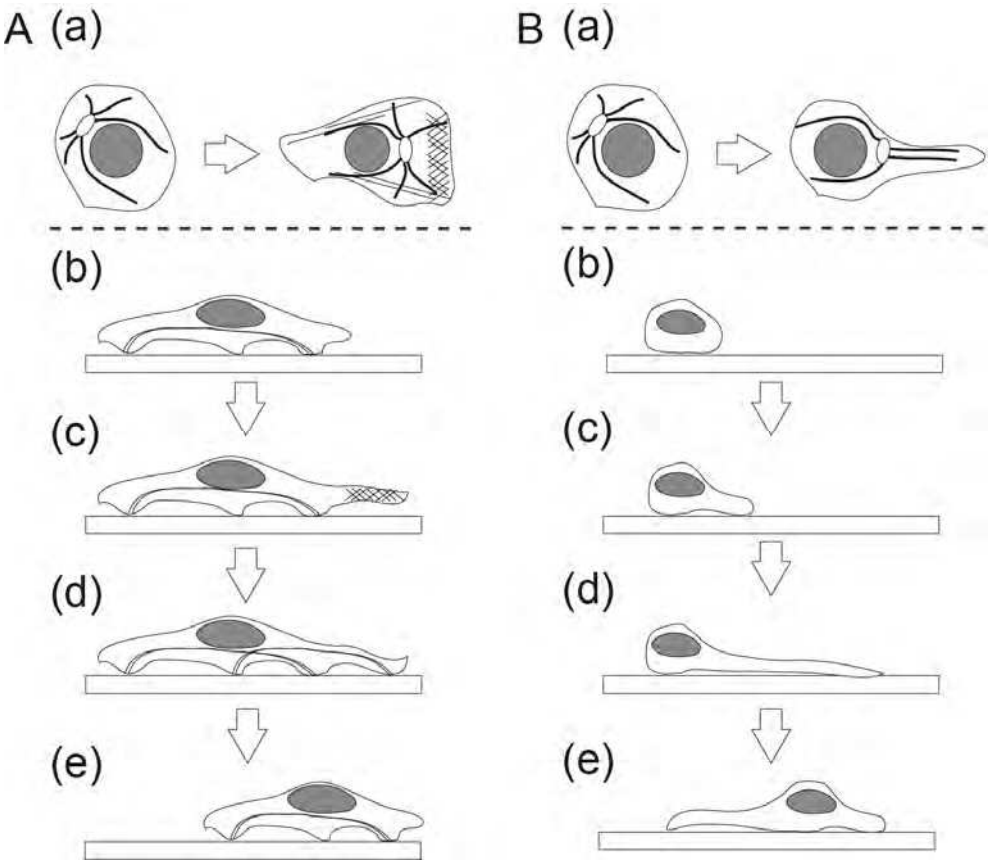

Fig. 2. Schematic illustration of adherent-cell migration integrating cytoskeleton and focal adhesion molecules complex, called as focal adhesions, in a fibroblast (A) and a neuronal cell (B) in overhead (a) and cross-sectional view (b–e). STEP 1 (a, b): Polarization of cell. The cell changes from a spherical or hemispherical shape to become anisotropic. STEP 2 (c): Generation of force driving the locomotion. Fibroblasts form filopodia and lamellipodia, and neuronal cells extend neurites. STEP 3 (d): Fixing the leading edge to the substrate. STEP 4 (e): Diving cell body.

However, there are common features in the movements of adherent cells and the basic mechanisms behind such movements are thought to be the same. Adherent cell migration is a result of the integrated dynamics of the cytoskeleton and adhesions molecules. The cytoskeleton runs throughout the cell body and acts as a "skeleton" and "motor" for the cell. The cytoskeleton is a protein complex composed of actin filaments, intermediate filaments, and microtubules. The cells adhere to the extracellular matrix, substrates, and other cells' surfaces with adhesion molecules including integrin to migrate, to survive, and to acquire extracellular information. The cells adhere to the extracellular matrix to transmit signals from extra-cell to intra-cell and vice versa. Therefore, adhesion to substrates through adhesions molecules acts as an "input–output adaptor". During migration, adherent cells kinetically anchor themselves to rearrange their cytoskeleton. Leading fronts, such as lamellipodia in fibroblasts and leading processes in neurons, are repeatedly formed through the extension of the cytoskeleton, which then adhere to the local environment. The posterior side of the cell is then released and retracted (Fig. 2). This process is being investigated by a number of research groups using a diverse range of observation methods because there are still many aspects that are not well understood(Smilenov et al. 1999; Flaherty et al. 2007; Hu et al. 2007).

1.1.3 Asymmetries in external stimuli determine migration direction

The migration direction of cells is determined by a number of different stimuli. The tendency of cells to change direction in response to the direction or gradient (spatial asymmetry) of external stimuli is known as *taxis*. The suffix "-taxis" is attached to prefixes representing specific stimuli to create words such as chemotaxis (movement in response to a chemical stimulus), magnetotaxis (magnetic stimulus), hydrotaxis (water), phototaxis (light), rheotaxis (water current), thermotaxis (temperature), and thigmotaxis (touch). Among the many types of taxes, chemotaxis, which is the tendency to move towards a higher or lower concentration of a specific chemical substance, is well known (Eisenbach et al. 2004). The tendency to move in the direction of a stimulus according to the gradient of the stimulus is known as positive taxis, and movement away from a stimulus is known as negative taxis. It is very important for bacteria, for example, to search for food (sugar) by swimming towards higher concentrations of food, and to avoid poison by swimming towards lower concentrations of poison. When social amoebae form fruiting bodies in response to a deteriorating environment, they are known to move towards higher concentrations of 3',5'-cyclic adenosine monophosphate (cyclic AMP) (Goldbeter 1996; Gregor et al. 2010). Even the cells of multicellular organisms display various taxes. For example, neurons extend neurites towards higher concentrations of netrin, with the cell body following suit and migrating in the same direction (Round and Stein 2007). Neutrophils, which are a type of white blood cell that eliminate invading bacteria by englobing them (phagocytosis), are capable of detecting very slight differences in concentration (1% difference between opposite sides of the neutrophil) of *N*-formyl-methionyl-leucylphenylalanine (FMLP), a protein derived from bacteria, and move towards higher FMLP concentrations (Weiner et al. 1999). Cells can thus change the direction of their movements according to asymmetries in external stimuli, and this ability is extremely important for survival.

1.2 Control of cell migration by using micropatterns

1.2.1 Cell migration around spatial obstacles

Much of the research on taxes of adherent cells described in the previous section is based on the results of observation under a microscope of cells adhering to a flat substrate applied to glass. However, in reality cells face a plethora of spatial obstacles (such as surrounding cells, soil and plants in nature, and bone and other connective tissues within the body) that make it difficult for them to migrate freely to their destinations (Fig. 1a). As such, cells need to not only recognize the surrounding geometry mechanically, but also process this geometrical information to determine the direction of their next migration (Ingber 2003). However, research on the relationship between geometry and cell migration has so far been limited. With respect to tactile sensibility (thigmesthesia), some animals are known to display the phenomenon of thigmotaxis, but this refers to the tendency of rats and other animals to hug the edges of walls and so forth when moving, and not to the kind of cell movements with respect to physical obstacles that we are discussing here. Investigation of how cells sense spatial restrictions and respond to them requires the creation of a geometry featuring various shapes on a cellular scale (several micrometres), but while structures on a millimetre scale can be crafted using apparatus such as ordinary lathes and milling machines, creation of structures on a smaller scale is difficult, and this is why research has been limited.

1.2.2 Micro-contact printing

Recent advances in photolithography and other microfabrication techniques have made it possible to create structures that are not toxic to cells and feature all kinds of cell-sized spatial patterns. Of these micropattern techniques, micro-contact printing, a soft lithography technique developed by Whitesides et al. at Harvard University, is particularly well-suited to small-scale research at universities, and is accordingly popular in the field of cellular engineering (Kumar and Whitesides 1993; Kane et al. 1999). Micro-contact printing involves first fabricating a finely patterned master that is then used to produce finely patterned stamps made of the thermosetting silicone elastomer polydimethylsiloxane (PDMS). The stamps are then used to print the patterns associated with cell adherence. Although making masters involves microfabrication techniques such photolithography that requires access to clean rooms and photolithographic equipment, masters do not have to be made in the place where subsequent processes are performed, and so can be made by other research laboratories or companies. Moreover, the subsequent processes can all be performed in a cellular biology laboratory, and enough micro-patterned culture vessels for several experiments can be produced with ease.

Extremely high precision masters can be created by using the silicon wafers that have become synonymous with semiconductor technologies. A technique using SU-8, a UV-curable resin, can be employed to make masters more easily (Ehrfeld et al. 1999). A spin coater is used to coat a silicon wafer or glass slide with a film of SU-8, and films with a thickness of several micrometres to over 100 μm can be created with excellent reproducibility. These thin SU-8 films are cured by irradiating with UV through a patterned mask, after which the uncured parts are washed away, leaving a three-dimensional pattern (Fig. 3a). SU-8 is not so strong, and thus the master can get chipped after repeated casting with PDMS, but because this method enables the production of micrometre-scale masters with high aspect ratios and low cell toxicity using relatively simple apparatus, it is very widely used.

After pouring the PDMS onto masters created in this way and curing at 60°C overnight, the microfabricated stamp can be removed from the master (Fig. 3b,c). Because PDMS keeps its shape very well on thermosetting, it is a superb material for reproducing sub-micrometre structures, and is also known to be non-toxic to cells. Also, PDMS is a pliant material, making it easy to remove stamps from the master and enabling good contact with the surface to be stamped to ensure even printing.

A substance for controlling cell adhesion is applied as "ink" to the PDMS stamp and then stamped onto the culture substrate (Fig. 3d,e). Whitesides et al. utilized self-assembled monolayers (SAMs) by printing with alkanethiol which have a variety of reactive functional groups to anchor cell adhesion related molecules, and created cell adhesion islands. There are two main micropatterning methods—printing with extracellular matrix substances that promote cell adhesion (e.g. collagen, fibronectin, laminin) (Scholl et al. 2000; Kaji et al. 2003; Hou et al. 2009), or printing with substances that impede cell adhesion (Yang et al. 2005; Saravia et al. 2007; Ohnuma et al. 2009). As we explain later, we used the latter method (Fig. 3f). Microfabrication techniques like these have enabled us to create cell adhesion patterns with a variety of geometric shapes and investigate the way that geometric patterns affect cell movement.

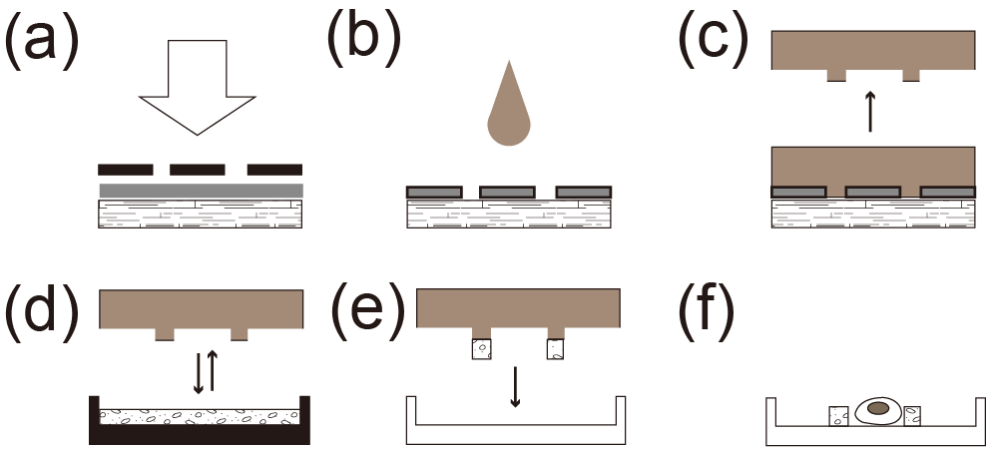

Fig. 3. Schematic illustration of micro-contact printing using a PDMS stamp: (a) fabricating a patterned master from a UV-curable resin by using UV-irradiation through a patterned mask, (b) applying silicone resin, (c) curing the resin to produce a patterned stamp, (d) dipping the patterned surface of the stamp in an ink, (e) printing the ink onto a culture dish, and (f) culturing the cells on the dish.

1.3 Biased movement in asymmetrical micropatterns

1.3.1 Asymmetry of cell shape and movement

How does the shape of the space in which a cell can move affect its movement? In the case of chemotaxis, the stimulating substance creates a concentration gradient (spatial asymmetry), and the cell moves in line with that concentration gradient (asymmetrical movement). From this, it is easy to suppose that the asymmetry of the space in which a

cell can move imparts a bias on the cell's movement. It is also known that the shape of cells that can move freely on a standard cell culture plate (one that enables cells to adhere and move uniformly) becomes asymmetric. For example, migrating fibroblasts are teardrop-shaped, with their front ends spreading out while the rear ends taper (Fig. 2a); migrating keratocytes, the epithelial cells of the epidermis of fish and frogs are half-moon-shaped (Svitkina et al. 1997; Keren et al. 2009) and neurons put out neurites in the direction in which they are moving (Fig. 2b) (Yamasaki et al. 2010). Adherent cells thus show a strong correlation between the direction of migration and the shape of the cell. Creating a cell adhesion island with an asymmetric geometry is accordingly likely to cause both cell shape and cell migration direction to become asymmetric in line with the asymmetry of the island (Fig. 1c,d). However, just as there are positive and negative taxes, spatial asymmetry needs to be actually measured to determine the direction in which it will bias cell movement.

1.3.2 Cells in a teardrop-shaped micropattern

With respect to the direction of cell movement and geometric pattern of cell adhesion sites, some very interesting research has been carried out on the teardrop shape that fibroblasts assume when moving through an unconfined space. In 2003, Brock et al. reported that cells confined within a polygonal shape tend to form lamellipodia at the corners of the polygon (Brock et al. 2003). Lamellipodia are formed when actin filaments create a mesh and the cell membrane advances, and tend to be formed at the fronts of cells when they are migrating (Fig. 2a). Fibroblasts in an unconfined space tend to advance with the blunt end of the teardrop to the front, but Brock et al.'s observations suggested that cells might also tend to advance towards sharp corners when geometrically confined.

In 2005, Jiang et al. published a very interesting paper on research to elucidate the relationship between cell shape and direction of movement (Jiang et al. 2005). They confined fibroblasts to a teardrop shape, and then used an electric pulse to release them from constraint and observed the direction in which they moved. They found that the fibroblasts moved in the direction of their blunt ends. This result suggested that the shape of the motile cell as a whole was a more important determinant of direction than the ease with which lamellipodia are formed at corners within the geometry. Following this, in 2007, Kumar et al. conducted observations on the direction taken by NIH3T3 fibroblasts in a chain of teardrop-shaped cell adhesion islands (Fig. 1d)(Kumar et al. 2007). They observed the direction of cell movements under various conditions, adjusting the arrangement of the teardrop islands, and the distance separating them, joining them in some cases, and leaving a gap of several micrometres in others, and so forth. They found that the direction of cell movements showed no bias towards either blunt end or sharp end of the teardrop-shaped islands, and that cells chose to move in whichever direction another cell adhesion island lay at the end of the longitudinal axis of each teardrop-shaped island. This suggested that the cell adhesion sites have a major effect on the direction of cell movement. In 2010, in experiments using epithelial cells, Kushiro et al. used the same teardrop-shaped cell adhesion island geometry as Kumar et al. to investigate how modifying the expression of the gene that controls the formation of lamellipodia affects cell movement (Kushiro et al. 2010). Unlike fibroblasts, epithelial cells moving in an unconfined space actively form broad lamellipodia at the front end of the

direction in which they are moving. Kushiro et al.'s findings regarding the direction of cell movement differed markedly from those of Kumar et al. for fibroblasts, but nevertheless showed that the direction of cell movement changes according to the degree of expression of the gene related to lamellipodia formation, and to the arrangement of teardrop-shaped islands and the distance between them.

1.3.3 Cells in ratchet-shaped micropattern

In 2009, Mahmud et al. investigated the same kind of movements using a slightly different geometry, one that used a ratchet-shaped micropattern (chained triangles connected in a linear manner) (Fig. 1c) rather than teardrop shapes as adhesion islands, and also included physical obstacles (Mahmud et al. 2009). They, too, observed bias in the direction of cell movement, and showed that this bias depends upon the type of cell involved. We explain in more detail later, but our group also created a geometrical pattern in which we combined triangles to form a ratchet shape. When we used this geometry to investigate the direction of movement of neuron-like cells, we observed a bias in movement and also found that the location at which the tips of neurites are formed is critical.

Some of these studies were conducted independently during much the same period. Conclusions that can be drawn from the above research using asymmetric geometrical patterns of about the same size as cells are: (1) many different cell types show bias in the direction of their movements; (2) the direction of cell movement changes according to the shape of the geometrical pattern in which cells can move, type of cell, and gene expression; and (3) bias in the direction of cell movement is related to the formation of lamellipodia and neurites, which are thought to be closely involved in cell movement. As such, while we can use geometrical patterns to bias the direction of cell movement, there is still much that we do not know about bias direction and the mechanisms involved in determining it.

1.3.4 Brownian ratchet theory

When discussing the bias direction of cell movement, we have not considered stochastic motion resulting from the spontaneous fluctuation of internal state of cell, which is known to be important in cell migrations (Oosawa 2001; Nakaoka et al. 2009). Here, we consider cell migration in an asymmetric geometrical pattern, taking stochastic motion into account. A Brownian particle, which exhibits stochastic motion due to thermal fluctuations, can be caused move directionally in a spatially asymmetric energy barrier under a non-equilibrium condition, as represented by a flashing ratchet (Fig. 4a)(Reimann 2002). In the case of so called rocking ratchet in which an oscillating force is applied to a Brownian particle (Fig. 4b), directional motion of the particle is also observed. This direction is known to be reversed by changes in the amplitude or period of the oscillating force (Bartussek et al. 1994; Reimann 2002). According to an experiment by Mahmud et al. (Mahmud et al. 2009), the transition probability for a cell in spatially asymmetric micropattern is described by a one-dimensional Brownian model. So, is there a possibility that the cause of the directional motion of a cell is analogous to that of Brownian ratchets? Furthermore, is a reversal of a cell's direction able to be observed when a signal is oscillating in the presence of an asymmetric geometry as is seen in the rocking ratchet (Fig. 4b)? It is up to future research to

determine whether cell migration is dictated by cell shape and/or by asymmetries in the surrounding space.

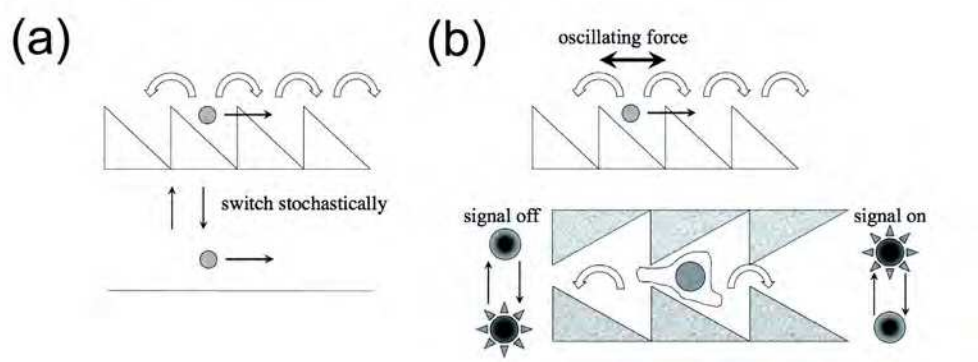

Fig. 4. Illustrated scheme of directional motion caused in a flashing ratchet (a) and in a rocking ratchet (b). In a flashing ratchet, a spatially asymmetric energy barrier, which a Brownian particle is subject to, switches in time stochastically. In a rocking ratchet, an oscillating force is applied to a Brownian particle besides the force exerted by a spatially asymmetric energy barrier.

1.4 Extrapolation to cell populations from single-cell migration analysis

Cells in multicellular organisms or wild environments are not independent entities and inevitably interact with the surrounding cells. Therefore, the effects of cell-to-cell interactions through physical contact, chemical signalling, nutrition competition, etc. must be considered to understand the roles of cellular movement and migration in natural contexts. Even if the movements of individual cells are characterized by simple rules, unexpected collective behaviours may emerge at the cell population level due to cell-to-cell interactions.

One of the most understood systems of collective migration is the fruiting-body formation of social amoebae. Upon starvation, thousands of individual cells co-ordinately migrate and aggregate to form fruiting bodies via signalling with cyclic AMP (Goldbeter 1996; Gregor et al. 2010). Fruiting bodies encapsulate spores that can survive severe stress environments for an extended period of time making such collective migration crucial for the survival of the species in harsh environments. Understanding the mechanism of this collective migration requires consideration of the effects of cell-to-cell interactions.

Collective migration also exists in bacteria. Lambert et al. developed a microfluidic device that allows the measurement of the efficiency of chemotactic migration by employing funnel-shaped barriers, and demonstrated that bacteria migrated by chemotaxis beyond the barriers to an area of higher nutrition only when cell density was high (Lambert et al. 2010). Another intriguing study on bacterial migration was reported by Park et al. in which they studied the time-evolution of spatial distributions of bacterial densities in a microfabricated maze (Park et al. 2003a; Park et al. 2003b). Despite the complex topology of the environment, the bacteria migrated and aggregated in a few confined position through chemotaxis

towards self-produced signals to create a high cell density. A high cell density is required for the formation of a biofilm, which is resistant to many kinds of stress. A biofilm is a bacterial community-based life-cycle mode that is known to contribute to the virulence of pathogens in bacterial infection (O'Toole et al. 2000; Lewis 2005).

When cells proliferate and divide during migration, another complexity arises. Differences between local environments generally induce different division rates in subpopulations. If subpopulations A and B in different locations have the division rates k_A and k_B ($>k_A$), the difference between the cell densities of the subpopulations grows with $\exp[(k_B - k_A)t]$, where t is time. Therefore, different division rates in a cell population can significantly affect the spatial distribution of cell density when the time-scale of observation is comparable to or longer than the mean doubling time of the cell population. This means that changes in spatial distribution cannot be attributed solely to the effect of cell migration. In phenomena such as embryogenesis or cancer metastasis, or during an immune response, cellular proliferation and migration proceed simultaneously. Cellular proliferation and migration are usually studied separately, but their coupling is an important subject for future research.

1.5 Biotechnological advantages of cell migration control by micropatterns

This kind of cell migration research is of course important from the life science perspective of elucidating the mechanisms behind fundamental cell functions, but it is also important from the perspective of applying the ability to control the direction of cell migration to cellular engineering and medical technology. As mentioned earlier, the migration of individual cells that make up multicellular organisms is a matter of great significance to the development and maintenance of functions of those organisms. The ability to control cell migration could lead to new or improved treatments for developmental disorders, tissue dysfunctions, healing of wounds, cancer metastasis, and so forth. Recently, the relationship between scaffold elasticity, which is closely related to cell migration, and the direction of differentiation has come to be discussed in relation to the induction of stem cell differentiation (Engler et al. 2006). Stem cells are known to be influenced by a huge number of endogenous factors (genes, RNAi, etc.) and exogenous factors such as chemicals and culture environment. The role that cell migration performs in differentiation is likely to attract growing interest.

The control of cell migration also has an important role to play in wound treatment and tissue regeneration (Friedl et al. 2004; Schneider et al. 2010). The migration not only of fibroblasts mentioned above, but also osteoblasts and osteoclasts in bone, and the cells involved in angiogenesis of blood vessels determine the form of those respective tissues, and are an important factor in the expression of the functions of those tissues.

Contributing to this kind of cellular engineering and medical treatment by equipping tissues with asymmetric spaces capable of controlling cell migration is indispensable to the further development of tailor-made treatment and advanced medical technology. Up to now, the focus of attention has been on cell adhesion substances that ensure that cells remain with and maintain the structure of the tissue to which they belong, but moving forward, development in the area of shaping spatial arrangements of those cells is likely to become an increasingly important endeavour.

2. Current studies on cell migration control by our group

2.1 Introduction

To determine the mechanisms of cell migration within cell-sized geometry, we focused on optically tracing two different types of cells that move in completely different manners. We used the rat adrenal pheochromocytoma cell line, PC12, and the fibroblast cell line, NIH3T3. Upon the addition of nerve growth factor, PC12 cells differentiate into sympathetic neuron-like cells with long, extended neurites (Greene et al. 1982; Ohnuma et al. 2006). Although PC12 cells migrate slowly, they are useful for the study of migration via long neurites. NIH3T3 cells are able to move very fast on glass slides and are frequently used as model cells in cell migration studies (Kumar et al. 2007).

To optically trace the migration of these cells in cell-sized geometry over a long period of time and to analyse the dynamics of cell–substrate contact sites (neurite tips for PC12 cells and focal adhesions for fibroblasts), which work both as input sensors for information regarding the local environment and as action sites for locomotion, we employed two different technologies. The first was a micro-contact printing technique using PDMS as the material for both the stamps and also the cell-repellent ink. We enable to keep the cells inside the PDMS micro-chamber for over 18 days (Ohnuma et al. 2009). The second technology was reflection interference contrast microscopy (RICM) (Curtis 1964). Using RICM, the distance between the glass and the cell surface membrane can be visualized as high-contrast images, which are images of the interference between the reflection of light off the glass–medium interface and off the medium–cell interface.

Analysis of the data showed that both PC12 and NIH3T3 exhibited biased migration in asymmetric micropatterns and that migration is likely to proceed from the sharp end of one micropattern unit to the blunt end of the adjacent micropattern unit. The contact sites of each migrating cell on the glass surface, however, expanded in both directions. The mechanism behind biased cell migration has still not been uncovered, but the current experimental setup will give us useful data for the control of cell migration.

2.2 PC12 migration control in a ratchet-shaped micropattern

2.2.1 Neuronal cell migration

Long-distance and directional migration of neuronal cells is a critical step in the developing and regenerating nervous system. Some neuronal cells migrate several millimetres to their final destinations. For example, inhibitory neurons originate in the ganglionic eminences, migrate radially to the cortex, and migrate parallel in the cortex surface to their destinations (Marín and Rubenstein 2001). The cellular mechanisms underlying these directional migratory activities have been extensively studied from the basis of chemotaxis (Ayala et al. 2007; Zheng and Poo 2007); however, chemokine gradients decrease with distance and determination of destination by each neuronal cell likely involves chemokine crosstalk. It was found that scaffolds, such as radial glia and blood vessels, provide routes that guide migrating neurons to their destinations (Rakic 1972; Gasser and Hatten 1990; Bovetti et al. 2007). These scaffolds act as “rails” to produce error-free, long-distance migration. However, physical and chemical circumstances, such as connective tissue, prevent cells from freely migrating towards their destination. Cells not only mechanically sense the local geometry,

but they also integrate this mechanical information into their migration (Ingber 2003). The relationship between geometry and cell migration has not been well studied.

2.2.2 Experimental set-up

To investigate the relationship between neuronal cells' local geometry and their migration, and, thus, uncover a potential control methodology, we performed a simple *in vitro* experiment (Ohnuma et al. 2009). We focused on periodic structures, which are abundant *in vivo*, by fabricating a ratchet-wheel shaped (gear-type) micropattern, which consists of a series of connected triangles. The micropattern was made by printing a PMDS film onto a collagen-coated culture dish (Fig. 3). Our working hypothesis was that neuronal cells would be able to migrate directionally on a periodic scaffold structure if the periodic unit was asymmetric. We tested the hypothesis using cultured PC12 cells that were attached only to the collagen-coated area between the core and the ratchet-shaped outer frame of the micro-chamber. Because the chamber was designed so that cell migration in the radial direction was restricted and the gap between the teeth and the core was equivalent to the size of a cell body, the cells migrated almost one-dimensionally in the tangential direction. We made both a left (L) and right (R) micropattern (Fig. 5a). The L and R chambers were line-symmetrical to one another and arranged alternately to serve as control chambers for one another. The core diameter, tooth depth, and gap between the teeth and core were approximately 100 μm, 40 μm, and 30 μm, respectively. PC12 cells were plated in neurite outgrowth medium including nerve growth factor in which the cells gradually extend long neurites over approximately 10 days (Greene et al. 1982; Ohnuma et al. 2006). It was previously reported that neuronal cells migrate following neurite extension (Hatten 2002), so it was expected that cell migration in the chambers would differ between cells with short neurites and cells with long neurites (Fig. 5b). Therefore, time-lapse micrographs of the cells in the micropattern were acquired twice: the first acquisition was for 70 hours starting from 1 day after plating, when the cells usually have short neurites; and the second acquisition was for 70 hours starting from 12 days after plating, when the cells usually have long neurites. We defined cell migration in the direction in which the ratchet teeth were tapered as positive migration (Fig. 5c).

2.2.3 Results and discussion

Using the PDMS printed L and R micropatterns, we found the same biased migration in both types of chambers. The PC12 cells in the L and R micropatterns migrated the same distance in a positive direction in both the first and second micrograph acquisition periods. These results suggested that the direction of migration is biased by chamber geometry, and supports our working hypothesis that the periodic nature of the asymmetrical scaffold determines migration direction. Next, we analysed the position of the cell body and the neurite tips because cell migration was strongly related to neurite formation. Although the mean length of the longest neurite was independent of microchamber geometry, the time-course trace of the neurite tips showed that they remained around the tips of the ratchet teeth. We also found that as cells migrated in a positive direction they tended to extend their neurites about one tooth ahead of the cell body and place the neurite tip at the tip of the tooth. The cell body then passed by the neurite tip as it migrated (Fig. 5c). It appears that PC12 cells use the neurite tips as a hook to "climb" the ratchet-shaped geometry. One

possible explanation for these results is that since the positively directed neurite bends along the tooth edge while the negatively directed neurite extends in a straight line, the tangential component of the maximal tension of the positive-direction neurite is higher than that of the negative-direction neurite. This results in the probability of continuous forward migration being higher than that of backward migration. Alternatively, the results can be described as thermally fluctuating spring-beads in a rocking ratchet (see 1.3.4).

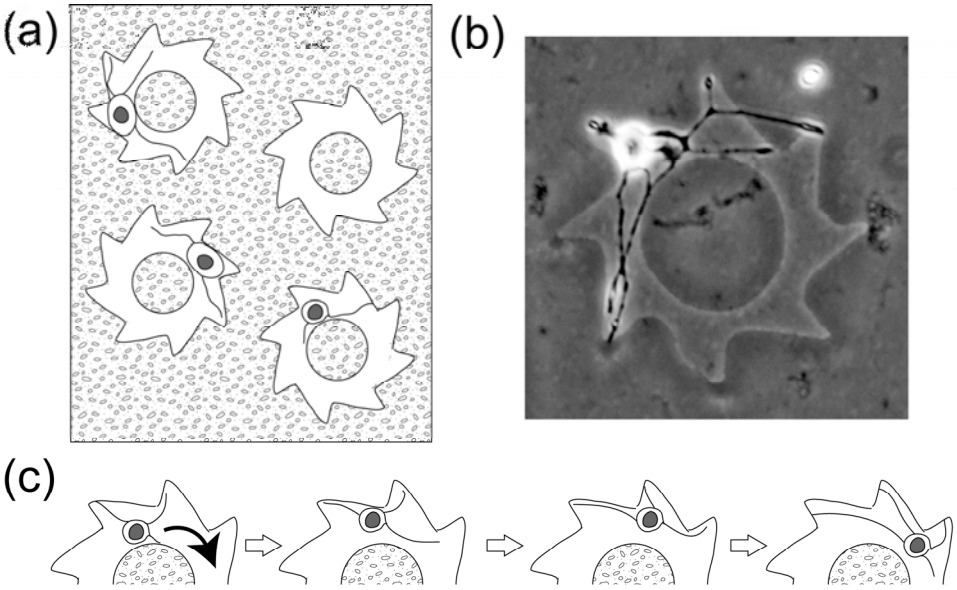

Fig. 5. Schematic illustration (a) and phase contrast microscopy image (b) of a PC12 cell extending its neurites in a gear-type micropattern. (c) The PC12 cell migrates in a positive direction (arrow).

2.3 Imaging of focal adhesions of NIH3T3 fibroblasts in a teardrop-type microchamber

2.3.1 Focal adhesions in cell migration

As previously mentioned, focal adhesions, which are membrane protein complexes, act as "input–output adaptors". Focal adhesions interact with substrates such as the extracellular matrix to allow the cell to adhere, migrate, and acquire extracellular information. Integrin is a major component of focal adhesions. Integrin is a family of trans-membrane proteins that connect physicochemically between the extracellular matrix and the cytoskeleton proteins inside of the cell to anchor cells and to transmit signals from extra-cell to intra-cell and vice versa. Outside-in signals, which are transmitted from outside to inside the cell, activate the adherent affinity of the complex, especially integrin. Inside-out signals via internal signal transduction proteins also activate integrin. Adherent affinity is regulated by both quantitative (density of the complex and ligand on the substrate) and qualitative properties (attractive interaction of the complex and the substrate). Mature focal complexes that have strongly adhered to a substrate are called focal adhesions.

Cell migration is the integrated dynamics of the cytoskeleton and focal adhesions, but how do cells actually migrate using this dynamics? The following is an accepted mechanism for the movement of adherent cells such as fibroblasts on substrates *in vitro*:

STEP 1: Polarization of cell. The shape of the cell changes from spherical or hemispherical to become anisotropic. Cdc42, PIP3, integrin, and microtubules are important in changing the shape of the cell. The cell membrane at the anterior part of the cell, the leading edge, starts to extend and produce actin filaments called filopodia.

STEP 2: Generation of force driving locomotion. Actin filaments are richly synthesized at the leading edge, and filopodia and lamellipodia are formed in association with the activation of Rac1, generating the force to extend the leading edge. The direction of the filopodia and lamellipodia is determined by the Rho protein family and/or the actin-binding protein family.

STEP 3: Fixing the leading edge to the substrate. At the leading edge, focal complexes are formed by the activation of integrin, which is stimulated by the inside-out and outside-in signals from both the cytoskeleton and the substrate. Focal complexes are also led by Rac1 and Cdc42 activity. The focal complexes mature to become focal adhesions at the leading edge. RhoA, which is activated at the posterior side of cell, is also involved in the formation of focal adhesions, so focal adhesions are also formed at the posterior side of the cell.

STEP 4: Diving cell body by generation of tension. Focal adhesions are the contact sites of the cytoskeleton and substrate outside of the cell. This becomes a point of tension through the cytoskeleton. The focal adhesions formed at the anterior and posterior sides are linked by the cytoskeleton as stress fibres force the cell body to shrink. The tension is regulated by actin motor proteins, myosin, and Rho-kinases (Smith et al. 2008).

STEP 5: Decomposition of the focal adhesions at the posterior side. Although the cell can decompose the focal adhesions at either the anterior or posterior side via RhoA, focal adhesion kinase, Src, or microtubules, the cell selects the focal adhesions at the posterior side for decomposition. This results in the posterior side of the cell shrinking due to the tension created by stress fibres in the cytoskeleton.

On the basis of these mechanisms, the physicochemistry of the migrating cell's surroundings potentially affects the migration direction. This aspect of cell migration should be investigated through the dynamics of the focal adhesions formed at both the anterior and posterior sides of the cell.

2.3.2 Imaging of focal adhesions of NIH3T3 fibroblasts using RICM

RICM can be used to observe focal adhesions. In the 1970s, electron microscopy revealed that the gap between cells and substrates was less than 30 nm (Abercrombie et al. 1971; Revel and Wolken 1973), however, the cells that were observed were fixed (not living) and the observation chamber was under a vacuum. Therefore, the development of optical microscopy for the observation of the focal adhesions of living cells was considered to be the next step. The principles of RICM were established by Curtis in 1964, (Curtis 1964) who regarded the medium between the glass and cell as a thin film, which allowed the observation of the distance between the glass and the cell surface as a high-contrast

images through the interference of light reflected from glass–medium and medium–cell membrane interfaces. The thickness of the thin medium "layer" is evaluated using the reflective index of the medium, cell membrane, and glass, and the wavenumber and angle of incident light (Bereiter-Hahn et al. 1979; Simson et al. 1998). Izzard and Lochner reported that the nearest distance between cell membranes and substrates is approximately 10 nm, which is shown as dark areas in RICM images. These are focal adhesions (or focal contacts) (Izzard and Lochner 1976). Sackmann et al. and others significantly developed RICM using a model cell membrane that is composed of giant vesicles bearing membrane protein or that has been modified with polymers (Bruinsma et al. 2000; Smith et al. 2008; Limozin and Sengupta 2009; Streicher et al. 2009). RICM has drawn much attention as a non-probing microscopy for observing focal adhesions (Yin et al. 2003; Sengupta et al. 2006; Théry et al. 2006).

Let us briefly summarize the principles of RICM (Fig. 6a). The intensities of the incident light, the light reflected from the interface of the medium and the surface of the glass substrate, and the light reflected from the interface of the medium and the cell membrane are depicted by I_0, I_{01}, and I_{12}, respectively. The intensity profile $I(x)$ of the interference between I_{01} and I_{12} is obtained by

$$I(x) = I_{01} + I_{02} + 2\left(I_{01}I_{02}\right)^{1/2}\left[2kh(x)\cos\theta + \delta\right] \tag{1}$$

where k is the wavenumber of light the phase of which is shifted with δ, and $h(x)$ is the distance of the cell membrane from the glass surface. Using the Fresnel equation with the reflection amplitude coefficients of each interface (r_{01}, r_{12}), I_{01} and I_{12} are substituted as follows: $I_{01} = r_{01}^2 I_0$ and $I_{12} = (1-r_{01}^2)r_{12}^2 I_0$. Therefore, the maximum (I_{max}) and minimum intensity (I_{min}) of the interference are obtained from $I_{max} = I_{01}+I_{12}+2(I_{01}I_{12})^{1/2}$ and $I_{min} = I_{01}+I_{12}-2(I_{01}I_{12})^{1/2}$, which allows the deduction of the following equation (n: refractive index):

$$h(x) = \frac{\lambda}{4\pi n}\left[\arccos\left\{\frac{2I(x)-(I_{max}+I_{min})}{I_{max}-I_{min}}\right\}+\delta\right] \tag{2}$$

When evaluating $h(x)$ of a living cell, the light distribution function should be included. The RICM pattern of the cell gives us the height of the cell membrane and the focal adhesions are the darkest areas with $m = 0$ in the following equation (λ: wavelength of light):

$$2kh(x)\cos\theta + \delta = \lambda m \,. \tag{3}$$

2.3.3 Experimental set-up

Since RICM requires a glass substrate surface, which is weakly cell-adherent, the high cell repellency of the micropattern becomes necessary. In order to obtain images of focal adhesions of cells migrating within micropatterns, we again adopted the micro-contact printing technique to construct a PDMS micropattern consisting of a series of connected teardrop shapes (Fig. 1d) (Kumar et al. 2007). The width of the neck between two teardrop shapes was about 6 μm, which was not notably larger than that of the actual design (5 μm). This convinces us that the current technique for constructing the micropattern worked well.

RICM was conducted with a halogen lamp with a 530 to 550 nm optical band-pass filter as the light source, two polarizing filters, and an objective lens with a quarter-wave plate. The microscope set-up was combined with a culture chamber managed by a temperature and humidity control box, and the cells were kept alive for several days in the chambers (Fig. 6b). This RICM set-up enables observation of the cells and the edges of micropatterns (Fig. 6d).

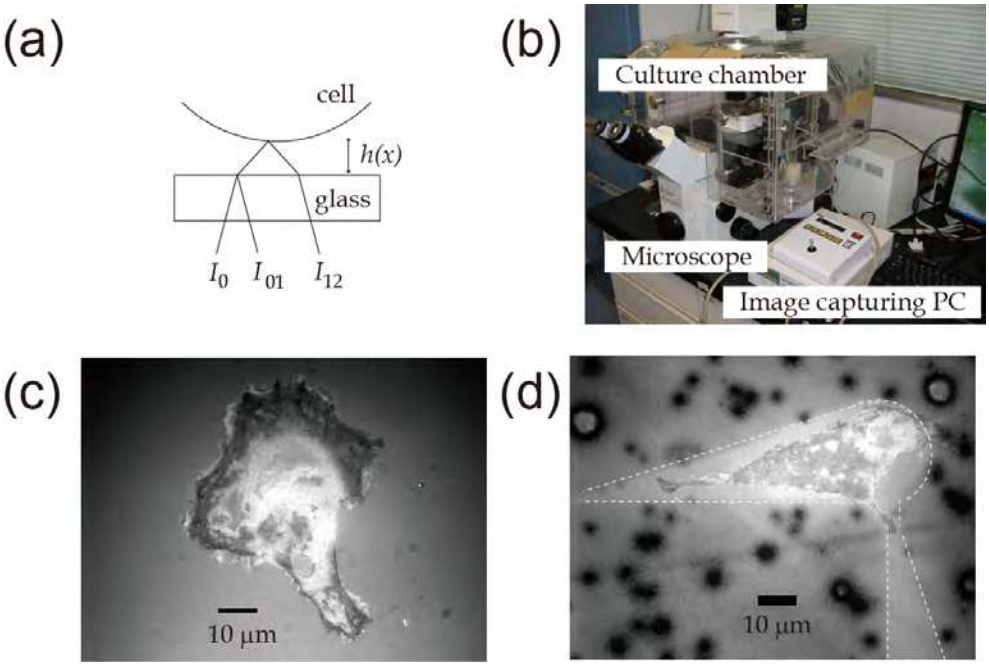

Fig. 6. (a) Schematic illustration of reflection interference contrast microscopy. (b) RICM set-up with a cell culturing system. (c,d) RICM images of NIH3T3 cultured on glass (c) or within the micropatterns (white dashed line) (d). Darkest areas in the images of NIH3T3 cell correspond to areas in most contact with the glass surface, i.e. focal adhesion.

2.3.4 Results and discussion

To validate the RICM set-up, we used a latex bead suspension as described (Rädler and Sackmann 1992; Rädler and Sackmann 1993; Kühner and Sackmann 1996; Heinrich et al. 2008). In brief, latex beads were suspended in 200 mM NaCl solution and then observed with the RICM set-up. Using equation (2), the heights of the beads from the glass surface were estimated to be about 10 nm. According to the Derjaguin–Landau–Verwey–Overbeek theory, the height of latex beads in a high ionic-strength suspension is several nanometers. Therefore, we deemed the performance of the RICM set-up to be sufficiently accurate. Immunofluorescence staining revealed that the dark spots in the RICM images were also areas of the cell that contained focal adhesion protein complex (Geiger 1979; Smilenov et al. 1999), suggesting that our RICM set-up was able to accurately visualize focal adhesions.

In RICM images, focal adhesions are dark and lamellipodia at the edge of cells are bright (Fig. 6c). Although the lamellipodia were extended onto the PDMS micropattern, they did not form focal adhesions, suggesting that the cells were restricted to the teardrop-shaped island in the PDMS micropattern. Formation and degradation of focal adhesions at the front and rear of migrating NIH3T3 fibroblasts in the teardrop-type micropattern (Fig. 1d) were clearly seen with our RICM set-up. Anticlockwise biased-migration of NIH3T3 cells in the teardrop-type micropattern was also observed (Figs. 1d, 6d), which was consistent with results previously reported (Kumar et al. 2007). The focal adhesions of each migrating cell in the micropattern, however, expanded both in clockwise and anticlockwise directions. The mechanism behind biased cell migration has still not been uncovered, but the PMDS micropattern and RICM set-up will give us useful data for the control of cell migration.

3. Conclusion

We have described an *in vitro* experimental model of cell migration guided by mechanical information of the local geometry. The fact that not only fibroblasts NIH3T3 but also neuronal PC12 cells, robustly exhibited biased movement within the micropatterns is indeed a surprise. Biased movement from the blunt end to the sharp end of the micropatterns resembles colloidal motion in a ratchet pattern. Further progress in the RICM imaging of focal adhesions will no doubt reveal the precise mechanism of cell migration and control within micropatterns.

Results from our group's study are expected to contribute the science of cell migration and the understanding of multi-cellular organisms. For example, the question of whether cell migration results from probabilistic (Brownian) or deterministic components of factors internal or external to cells will be solved when the current set-up is combined with fluorescent microscopy and protein-specific probes. The resultant knowledge on cell migration may also stimulate the research field of soft micromachines which can involve sensory motor coupling (Borckmans et al. 2009; Toyota et al. 2009; Masubuchi et al. 2011). At present, there is no evidence that scaffolds with asymmetrical surface structures exist *in vivo*. However, both repetitive structures, including the somite and the cortical layer, and asymmetric protein distributions are abundant *in vivo*. These studies offer new insights into the migration of cells controlled by mechanical stimulation and suggest strategies for designing artificial scaffolds that direct cell migration.

4. Acknowledgments

Mr Tomohiro Nakanishi (Chiba University), Prof. Masanori Fujinami (Chiba University) and Prof. Makoto Asashima (The University of Tokyo, National Institute of Advanced Industrial Science and Technology) are acknowledged for the discussion on the mechanism of cell migration, the micropattern manufacturing, and the optical setups. TT was financially supported by the Izumi Science and Technology Foundation and by a Grant-in-Aid for Scientific Research (Young Scientist B) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. KO was financially supported by the Program to Disseminate Tenure Tracking System from the Japan Science and Technology Agency. TT and KO were financially supported by a Grant-in-Aid for Scientific Research on Priority Areas "System cell engineering by multi-scale manipulation" (20034015) from MEXT, Japan.

5. References

Abercrombie, M., Heaysman, J. E. & Pegrum, S. M. (1971). *The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella*, Exp Cell Res, Vol. 67, No. 2, pp. 359-367

Ayala, R., Shu, T. & Tsai, L. H. (2007). *Trekking across the brain: the journey of neuronal migration*, Cell, Vol. 128, No. 1, pp. 29-43

Bartussek, R., Hanggi, P. & Kissner, J. G. (1994). *Periodically Rocked Thermal Ratchets*, Europhys Lett, Vol. 28, No. 7, pp. 459-464

Bereiter-Hahn, J., Fox, C. H. & Thorell, B. O. (1979). *Quantitative reflection contrast microscopy of living cells*, J Cell Biol, Vol. 82, No. 3, pp. 767-779

Borckmans, P., De Kepper, P. & Khokhlov, A. R. (2009). Chemomechanical Instabilities in Responsive Materials, ISBN 9048129923, Springer Verlag

Bovetti, S., Hsieh, Y. C., Bovolin, P., Perroteau, I., Kazunori, T. & Puche, A. C. (2007). *Blood vessels form a scaffold for neuroblast migration in the adult olfactory bulb*, J Neurosci, Vol. 27, No. 22, pp. 5976-5980

Bray, D. (2001). Cell Movements: From Molecules to Motility, I SBN 0815332823, Garland Publishing

Brock, A., Chang, E., Ho, C. C., LeDuc, P., Jiang, X., Whitesides, G. M. & Ingber, D. E. (2003). *Geometric determinants of directional cell motility revealed using microcontact printing*, Langmuir, Vol. 19, No. 5, pp. 1611-1617

Bruinsma, R., Behrisch, A. & Sackmann, E. (2000). *Adhesive switching of membranes: experiment and theory*, Phys Rev E, Vol. 61, No. 4, pp. 4253-4267

Curtis, A. S. (1964). *The Mechanism of Adhesion of Cells to Glass. A Study by Interference Reflection Microscopy*, J Cell Biol, Vol. 20, pp. 199-215

Ehrfeld, W., Hessel, V., L we, H., Schulz, C. & Weber, L. (1999). *Materials of LIGA technology*, Microsystem technologies, Vol. 5, No. 3, pp. 105-112

Eisenbach, M., Lengeler, J. W. & Varon, M. (2004). Chemotaxis: A New Approach to Global Strategy and Leadership, I SBN 1860944132, Imperial College Pr

Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. (2006). *Matrix elasticity directs stem cell lineage specification*, Cell, Vol. 126, No. 4, pp. 677-689

Flaherty, B., McGarry, J. P. & McHugh, P. E. (2007). *Mathematical models of cell motility*, Cell Biochem Biophys, Vol. 49, No. 1, pp. 14-28

Friedl, P., Hegerfeldt, Y. & Tusch, M. (2004). *Collective cell migration in morphogenesis and cancer*, Int J Dev Biol, Vol. 48, pp. 441-450

Gasser, U. E. & Hatten, M. E. (1990). *Central nervous system neurons migrate on astroglial fibers from heterotypic brain regions in vitro*, Proc Natl Acad Sci U S A, Vol. 87, No. 12, pp. 4543-4547

Geiger, B. (1979). *A 130K protein from chicken gizzard: its localization at the termini of microfilament bundles in cultured chicken cells*, Cell, Vol. 18, No. 1, pp. 193-205

Goldbeter, A. (1996). Biochemical oscillations and cellular rhythms: the molecular bases of periodic and chaotic behaviour, I, Cambridge University. Cambridge. GB

Greene, L. A., Burstein, D. E. & Black, M. M. (1982). *The role of transcription-dependent priming in nerve growth factor promoted neurite outgrowth*, Dev Biol, Vol. 91, No. 2, pp. 305-316

Gregor, T., Fujimoto, K., Masaki, N. & Sawai, S. (2010). *The onset of collective behavior in social amoebae*, Science, Vol. 328, No. 5981, pp. 1021-1025

Hatten, M. E. (2002). *New directions in neuronal migration*, Science, Vol. 297, No. 5587, pp. 1660-1663

Heinrich, V., Wong, W. P., Halvorsen, K. & Evans, E. (2008). *Imaging biomolecular interactions by fast three-dimensional tracking of laser-confined carrier particles*, Langmuir, Vol. 24, No. 4, pp. 1194-1203

Hou, S., Li, X. X., Li, X. Y., Feng, X. Z., Guan, L., Yang, Y. L. & Wang, C. (2009). *Patterning of 293T fibroblasts on a mica surface*, Anal Bioanal Chem, Vol. 394, No. 8, pp. 2111-2117

Hu, K., Ji, L., Applegate, K. T., Danuser, G. & Waterman-Storer, C. M. (2007). *Differential transmission of actin motion within focal adhesions*, Science, Vol. 315, No. 5808, pp. 111-115

Ingber, D. E. (2003). *Tensegrity I. Cell structure and hierarchical systems biology*, J Cell Sci, Vol. 116, No. Pt 7, pp. 1157-1173

Izzard, C. S. & Lochner, L. R. (1976). *Cell-to-substrate contacts in living fibroblasts: an interference reflexion study with an evaluation of the technique*, J Cell Sci, Vol. 21, No. 1, pp. 129-159

Jiang, X., Bruzewicz, D. A., Wong, A. P., Piel, M. & Whitesides, G. M. (2005). *Directing cell migration with asymmetric micropatterns*, Proc Natl Acad Sci U S A, Vol. 102, No. 4, pp. 975-978

Kühner, M. & Sackmann, E. (1996). *Ultrathin hydrated dextran films grafted on glass: preparation and characterization of structural, viscous, and elastic properties by quantitative microinterferometry*, Langmuir, Vol. 12, No. 20, pp. 4866-4876

Kaji, H., Takoh, K., Nishizawa, M. & Matsue, T. (2003). *Intracellular Ca^{2+} imaging for micropatterned cardiac myocytes*, Biotech Bioeng, Vol. 81, No. 6, pp. 748-751

Kane, R. S., Takayama, S., Ostuni, E., Ingber, D. E. & Whitesides, G. M. (1999). *Patterning proteins and cells using soft lithography*, Biomaterials, Vol. 20, No. 23-24, pp. 2363-2376

Keller, P. J., Schmidt, A. D., Wittbrodt, J. & Stelzer, E. H. (2008). *Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy*, Science, Vol. 322, No. 5904, pp. 1065-1069

Keren, K., Yam, P. T., Kinkhabwala, A., Mogilner, A. & Theriot, J. A. (2009). *Intracellular fluid flow in rapidly moving cells*, Nat Cell Biol, Vol. 11, No. 10, pp. 1219-1224

Kumar, A. & Whitesides, G. M. (1993). *Features of gold having micrometer to centimeter dimensions can be formed through a combination of stamping with an elastomeric stamp and an alkanethiol ''ink''followed by chemical etching*, App Phys Lett, Vol. 63, No. 14, pp. 2002-2004

Kumar, G., Ho, C. C. & Co, C. C. (2007). *Guiding cell migration using one-way micropattern arrays*, Adv Mater, Vol. 19, No. 8, pp. 1084-1090

Kushiro, K., Chang, S. & Asthagiri, A. R. (2010). *Reprogramming directional cell motility by tuning micropattern features and cellular signals*, Adv Mater, Vol. 22, No. 40, pp. 4516-4519

Lambert, G., Liao, D. & Austin, R. H. (2010). *Collective Escape of Chemotactic Swimmers through Microscopic Ratchets*, Phys Rev Lett, Vol. 104, No. 16, pp. 168102

Lewis, K. (2005). *Persister cells and the riddle of biofilm survival*, Biochemistry (Moscow), Vol. 70, No. 2, pp. 267-274

Limozin, L. & Sengupta, K. (2009). *Quantitative reflection interference contrast microscopy (RICM) in soft matter and cell adhesion*, ChemPhysChem, Vol. 10, No. 16, pp. 2752-2768

Mahmud, G., Campbell, C. J., Bishop, K. J. M., Komarova, Y. A., Chaga, O., Soh, S., Huda, S., Kandere-Grzybowska, K. & Grzybowski, B. A. (2009). *Directing cell motions on micropatterned ratchets*, Nature Phys, Vol.5, 606-612

Marín, O. & Rubenstein, J. L. (2001). *A long, remarkable journey: tangential migration in the telencephalon*, Nat Rev Neurosci, Vol. 2, No. 11, pp. 780-790

Masubuchi, M., Toyota, T., Yamada, M. & Seki, M. (2011). *Fluidic shear-assisted formation of actuating multilamellar lipid tubes using microfabricated nozzle array device*, Chem Comm, Vol. 47, 8433-8435

Nakaoka, Y., Imaji, T., Hara, M. & Hashimoto, N. (2009). *Spontaneous fluctuation of the resting membrane potential in Paramecium: amplification caused by intracellular Ca^{2+}*, J Exp Biol, Vol. 212, No. 2, pp. 270-276

O'Toole, G., Kaplan, H. B. & Kolter, R. (2000). *Biofilm formation as microbial development*, Ann Rev Microbiol, Vol. 54, No. 1, pp. 49-79

Ohnuma, K., Hayashi, Y., Furue, M., Kaneko, K. & Asashima, M. (2006). *Serum-free culture conditions for serial subculture of undifferentiated PC12 cells*, J Neurosci Methods, Vol. 151, No. 2, pp. 250-261

Ohnuma, K., Toyota, T., Ariizumi, T., Sugawara, T. & Asashima, M. (2009). *Directional migration of neuronal PC12 cells in a ratchet wheel shaped microchamber*, J Biosci Bioeng, Vol. 108, No. 1, pp. 76-83

Oosawa, F. (2001). *Spontaneous signal generation in living cells*, Bull Math Biol, Vol. 63, No. 4, pp. 643-654

Park, S., Wolanin, P. M., Yuzbashyan, E. A., Lin, H., Darnton, N. C., Stock, J. B., Silberzan, P. & Austin, R. (2003a). *Influence of topology on bacterial social interaction*, Proc Natl Acad Sci U S A, Vol. 100, No. 24, pp. 13910-13915

Park, S., Wolanin, P. M., Yuzbashyan, E. A., Silberzan, P., Stock, J. B. & Austin, R. H. (2003b). *Motion to form a quorum*, Science, Vol. 301, No. 5630, pp. 188

Rädler, J. & Sackmann, E. (1992). *On the measurement of weak repulsive and frictional colloidal forces by reflection interference contrast microscopy*, Langmuir, Vol. 8, No. 3, pp. 848-853

Rädler, J. & Sackmann, E. (1993). *Imaging optical thicknesses and separation distances of phospholipid vesicles at solid surfaces*, Journal de Physique II, Vol. 3, No. 5, pp. 727-748

Rakic, P. (1972). *Mode of cell migration to the superficial layers of fetal monkey neocortex*, J Comp Neurol, Vol. 145, No. 1, pp. 61-83

Reimann, P. (2002). *Brownian motors: noisy transport far from equilibrium*, Phys Reports, Vol. 361, No. 2-4, pp. 57-265

Revel, J. P. & Wolken, K. (1973). *Electronmicroscope investigations of the underside of cells in culture*, Exp. Cell Res, Vol. 78, pp. 1-14

Round, J. & Stein, E. (2007). *Netrin signaling leading to directed growth cone steering*, Current opinion in neurobiology, Vol. 17, No. 1, pp. 15-21

Saravia, V., Kupcu, S., Nolte, M., Huber, C., Pum, D., Fery, A., Sleytr, U. B. & Toca-Herrera, J. L. (2007). *Bacterial protein patterning by micro-contact printing of PLL-g-PEG*, J Biotech, Vol. 130, No. 3, pp. 247-252

Schneider, L., Cammer, M., Lehman, J., Nielsen, S. K., Guerra, C. F., Veland, I. R., Stock, C., Hoffmann, E. K., Yoder, B. K. & Schwab, A. (2010). *Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts*, Cell Physiol Biochem, Vol. 25, No. 2-3, pp. 279-292

Scholl, M., Sprossler, C., Denyer, M., Krause, M., Nakajima, K., Maelicke, A., Knoll, W. & Offenhausser, A. (2000). *Ordered networks of rat hippocampal neurons attached to silicon oxide surfaces*, J Neurosci Methods, Vol. 104, No. 1, pp. 65-75

Sengupta, K., Aranda-Espinoza, H., Smith, L., Janmey, P. & Hammer, D. (2006). *Spreading of neutrophils: from activation to migration*, Biophys J, Vol. 91, No. 12, pp. 4638-4648

Simson, R., Wallraff, E., Faix, J., Niew hner, J., Gerisch, G. & Sackmann, E. (1998). *Membrane bending modulus and adhesion energy of wild-type and mutant cells of Dictyostelium lacking talin or cortexillins*, Biophys J, Vol. 74, No. 1, pp. 514-522

Smilenov, L. B., Mikhailov, A., Pelham, R. J., Marcantonio, E. E. & Gundersen, G. G. (1999). *Focal adhesion motility revealed in stationary fibroblasts*, Science, Vol. 286, No. 5442, pp. 1172-1174

Smith, A. S., Sengupta, K., Goennenwein, S., Seifert, U. & Sackmann, E. (2008). *Force-induced growth of adhesion domains is controlled by receptor mobility*, Proc Natl Acad Sci U S A, Vol. 105, No. 19, pp. 6906-6911

Streicher, P., Nassoy, P., Barmann, M., Dif, A., Marchi-Artzner, V., Brochard-Wyart, F., Spatz, J. & Bassereau, P. (2009). *Integrin reconstituted in GUVs: A biomimetic system to study initial steps of cell spreading*, Biochim Biophys Acta (BBA)-Biomembranes, Vol. 1788, No. 10, pp. 2291-2300

Svitkina, T. M., Verkhovsky, A. B., McQuade, K. M. & Borisy, G. G. (1997). *Analysis of the actin-myosin II system in fish epidermal keratocytes: mechanism of cell body translocation*, J Cell Biol, Vol. 139, No. 2, pp. 397-415

Théry, M., P pin, A., Dressaire, E., Chen, Y. & Bornens, M. (2006). *Cell distribution of stress fibres in response to the geometry of the adhesive environment*, Cell motility and the cytoskeleton, Vol. 63, No. 6, pp. 341-355

Toyota, T., Maru, N., Hanczyc, M. M., Ikegami, T. & Sugawara, T. (2009). *Self-propelled oil droplets consuming "fuel" surfactant*, J Am Chem Soc, Vol. 131, No. 14, pp. 5012-5013

Weiner, O. D., Servant, G., Welch, M. D., Mitchison, T. J., Sedat, J. W. & Bourne, H. R. (1999). *Spatial control of actin polymerization during neutrophil chemotaxis*, Nature cell biology, Vol. 1, No. 2, pp. 75-81

Yamasaki, E., Tanaka, D. H., Yanagawa, Y. & Murakami, F. (2010). *Cortical GABAergic interneurons transiently assume a Sea Urchin-like nonpolarized shape before axon initiation*, J Neurosci, Vol. 30, No. 45, pp. 15221-15227

Yang, I. H., Co, C. C. & Ho, C. C. (2005). *Alteration of human neuroblastoma cell morphology and neurite extension with micropatterns*, Biomaterials, Vol. 26, No. 33, pp. 6599-6609

Yin, C., Liao, K., Mao, H. Q., Leong, K. W., Zhuo, R. X. & Chan, V. (2003). *Adhesion contact dynamics of HepG2 cells on galactose-immobilized substrates*, Biomaterials, Vol. 24, No. 5, pp. 837-850

Zheng, J. Q. & Poo, M. M. (2007). *Calcium signaling in neuronal motility*, Annu Rev Cell Dev Biol, Vol. 23, pp. 375-404

Innovations in Biotechnology

Edited by Dr. Eddy C. Agbo

ISBN 978-953-51-0096-6
Hard cover, 474 pages
Publisher InTech
Published online 17, February, 2012
Published in print edition February, 2012

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