7

# Cell Migration

And their feet move
rhythmically ...
Sappho

I thought I could see a kind of motion ahead of me.
Joseph Conrad, *Heart of Darkness*

Cell migration is crucial to the life of unicellular and multicellular organisms. Unicellular organisms migrate to find food and avoid predators; this migration can occur by swimming through a fluid, which is achieved by flagellar or ciliary beating (exemplified by *E. coli* or *Paramecium*, respectively), or crawling along a surface (as in amoebae). In multicellular organisms, migration of a particular cell population is often a component of complex multicellular behaviors including tumor invasion and metastasis [1], embryogenesis [2], angiogenesis [3], and immune responses [4] (Figure 7.1 and Table 7.1). In both cases, the speed and pattern of migration are determined by the nature of the cell and by chemicals (both soluble and surface-bound) in the environment. Since cell migration is critical in the formation or regeneration of tissues, a clearer understanding of the dynamics of cell migration would greatly enhance our ability to design materials and processes for tissue engineering.

## 7.1 Overview of Cell Migration

Cell migration is a fundamental mechanism for forming of structures within developing embryos. Accordingly, the migration of cells during embryogenesis is under exquisite control; development of tissue structure and cell migration are interdependent. Chapter 3 discussed limb regeneration in salamanders, a process in which positional gradients of cell adhesion influence cell migration and, ultimately, tissue structure. Similarly, the rate and migration of myogenic cells from the somitic mesoderm is influenced by the presence of local signals in the form of diffusible factors and extracellular matrix composition. These local signals are also produced by cells, with the result that cells throughout the developing limb (which are present in a particular arrangement or tissue structure) control and coordinate the migration of myogenic cells by secretion of activating factors and expression or organization of extracellular matrix molecules [5]. A better understanding of the mechanisms underlying cell migration in the embryo, and the strategies that nature uses to control migration, will almost certainly provide inspiration for tissue engineering.

Cell migration underlies many important physiological functions in adults. For example, the immune system, with its widely dispersed ensemble of B and T cells, relies heavily on the coordinated migration of individual cells to patrol the body and to provide opportunities for cell–cell interaction. Cell migration is an essential—and perhaps unavoidable—consequence of treatments that depend on transplantation of cells or tissues. A recent study of magnetically





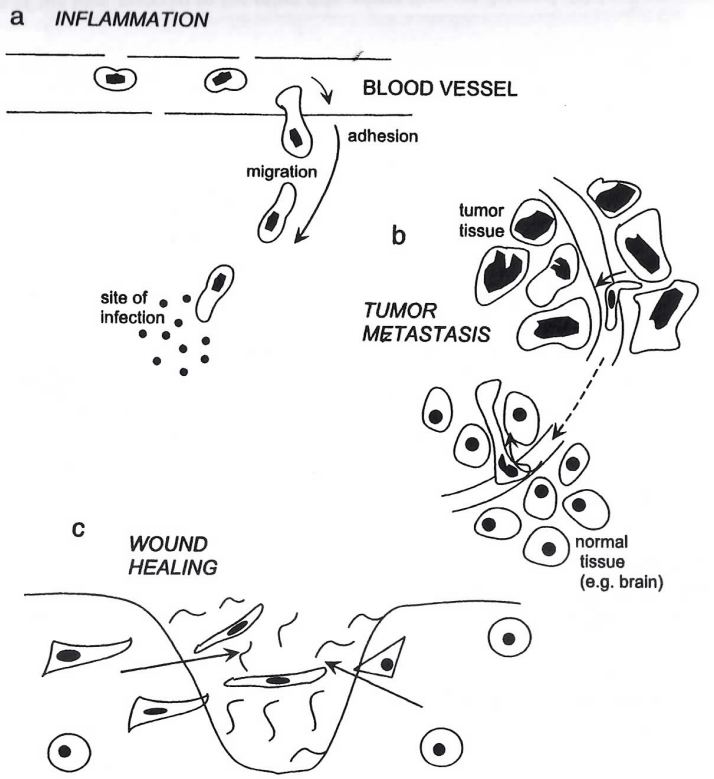


Figure 7.1. Cell migration is an essential step in many physiological processes. (a) Neutrophils transmigrate through the blood vessel wall and invade the tissue space; (b) tumor cells in the circulation can migrate into tissues distant from the original tumor site; (c) fibroblasts migrate into wound sites to initiate healing.

Table 7.1
Importance of Cell Migration

| Type of Migrating Cell | Role of Migrating Cell |
|---|---|
| Neutrophils | Phagocytosis of bacteria |
| Lymphocytes | Destruction of infected cells |
| Macrophages | Antigen presentation |
| Endothelial cells | Angiogenesis |
| Epidermal cells and fibroblasts | Wound healing |
| Tumor cells | Metastasis |
| Neurons and axons | Development and regeneration in the nervous system |
| Embryonic cells | Embryogenesis |

Many cells in the body are motile. Several cells types are listed above, together with their role in human physiology.

labeled oligodendrocyte progenitors by magnetic resonance imaging (MRI) demonstrated that cells migrate to cover a region > 8 mm in size during the first 10 days after injection into the spinal cord [6]. Some cell migration is desirable in many situations, such as this example of cell transplantation within the spinal cord; dispersion of cells within the nervous system leads to distribution of their capacity to myelinate. However, cell migration may cause side effects or loss of function at the transplantation site. In the neurotransplantation example, where cells are intended to function locally in the tissue, migration is counterproductive if all of the transplanted cells leave the local site. In the more general case, cell migration to distant sites after organ transplantation may lead to microchimerism (see Section 10.3)—the appearance of cells from the transplanted tissue at low levels in organs throughout the patient—that influences subsequent immune responses to the transplanted tissue.

In summary, cell migration enables normal fetal development and circulation of certain cells to occur throughout life. In tissue engineering, cell migration may be desirable or dangerous, depending on the application; in either case, our ability to understand and manipulate cell migration will be a critical component of our ultimate success.

## 7.2 Experimental Observations on Cell Motility

### 7.2.1 Common Experimental Techniques

The physiological significance of cell migration has motivated numerous experimental studies [7, 8], beginning with the seminal time-lapse studies of Comandon [9]. In general, structural complexity and lack of transparency make it difficult to study migration of cells within multicellular organisms. However, modern imaging methods can permit observation of cell movement within living tissues. Fluorescent dyes can be used to label cells, which can then be tracked by time-lapse microscopy [10–12] or conventional microscopy after sectioning [13] or collection of tissue samples [14]. New microscopy techniques—including two-photon imaging [15, 16]—are enabling the visualization of structures that were previously difficult to image within thick specimens. Green fluoresent proteins (GFPs) can be introduced into specific cell populations to permit tracking of cell lineages during development and migration [10]. Lymphocytes and other phagocytic cells will ingest superparamagnetic particles, enabling visualization of cell position within the body by magnetic resonance imaging [17]. MRI techniques can now be performed with sufficient resolution (~ 1 μm) to allow tracking of cell movements during embryogenesis [18] or after transplantation [6]. Immunological or genetic markers—either natural [14, 19] or introduced by transfection [20]—can be introduced into cells for tracking; this is usually accomplished by sectioning tissue to identify the position of the marked cell. When most of these techniques are applied in animals, the movement of a sub-population of labeled cells is recorded; to date, most of these analyses have been descriptive. Time-lapse techniques permit quantitative analysis of individual cell movements; these continuous records of cell movement in the native tissue are providing insight into the mechanisms of cell migration.

Historically, most studies of cell migration involve cell culture methods that attempt to replicate specific aspects of the natural cell environment. Most experimental methods for characterizing cell motility can be placed into one of two categories (Figure 7.2). In visual assays or single-cell assays, the movements of a small number of cells are observed individually [21–23]. Population techniques involve the observation of the collective movements of a group of

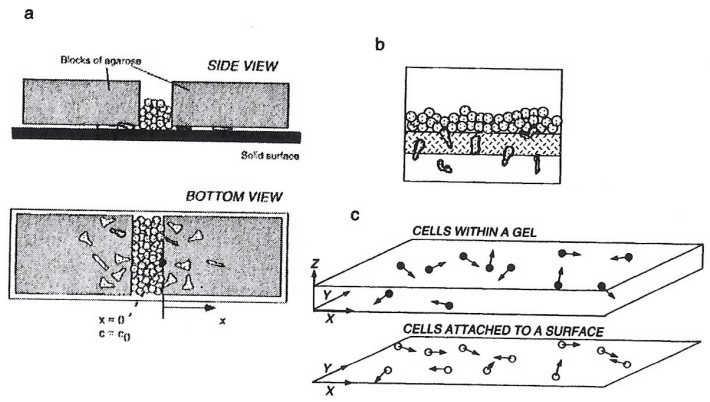


Figure 7.2. Schematic diagram of common cell migration assay systems. (a) In the under-agarose assay, a cell suspension is placed in a well of semisolid agarose; motile cells crawl on the solid substrate underneath the agarose. (b) In the filter assay, a cell suspension is placed on a filter with small pores; motile cells crawl through the pores of the filter material to the other side, where they are detected. (c) In direct visualization assays, the paths of movement of many individual cells are directly observed for cells migrating on surfaces and within solid gels.

cells [24–26]. The experimental details, advantages, and limitations of these assays have been reviewed [27]. In both measurements, cell motility can be quantified in terms of intrinsic cell motility parameters [22, 28–31], which are described in more detail in the sections that follow. One such parameter, the random motility coefficient ($\mu$), characterizes the migration of cells in isotropic environments. Although each individual technique is self-consistent and reproducible, experimentally measured motility coefficients can vary significantly between different assay systems [30]. This variation suggests that methods that mimic the host environment may be most useful for predicting cell behavior *in vivo*.

The simplest and most commonly used migration assays present cells with an environment that is significantly different from that encountered by cells in an organism. For example, many assay systems require cells to be attached to a two-dimensional substrate, while in tissues motile cells are frequently dispersed in three dimensions. Also, in most assays, motile cells contact glass, tissue culture polystyrene, cellulose ester, or cellulose acetate filters; the native extracellular matrix (ECM), however, is a complex gel of biopolymers, with collagen being the most abundant.

In recent years, three-dimensional cell culture techniques have been developed to more closely simulate tissues [32–36]. Of the few studies that have addressed cell motility in these situations [37–42], most have involved plating a cell suspension on top of a collagen gel and subsequently following the infiltration of the gel by the cells. Most typically, infiltration is measured by following the leading front distance of the cell population as a function of time. While much information can be gained from these studies, the time course of infiltration is usually on the order of hours or days. Furthermore, measurement of the leading front distance provides an accurate description of the behavior of the fastest moving cells rather than the entire cell population.

### 7.2.2 Cell Polarity

Migrating cells in culture look as if they are moving in a particular direction (Figure 7.3). The characteristic shape—which depends on cell type—is due to the internal arrangement of cytoskeletal components, the pattern of adhesion sites between the cell and the surface, and the movement of membranes around the cell periphery (Figure 7.4). The "front" region of the cell (that is, the edge that faces the direction of cell movement) is called the leading edge or lamellipodium and the "rear" region is called the uropodium. Both of these regions are relatively stable during periods when the cell is moving forward in a straight line, without turning; this period, which is called the persistence time $P$, is critical to the overall pattern of movement of the cell. The development of a polarized morphology, with a front and rear region that are morphologically distinct (Figure 7.3) and biochemically distinct (Figure 7.4), is an early event in the migration of many cells, including leukocytes [43].

Cell movement is initiated by active membrane protrusion in the lamellipodial region. In fibroblasts, the leading edge of the cell protrudes and retracts





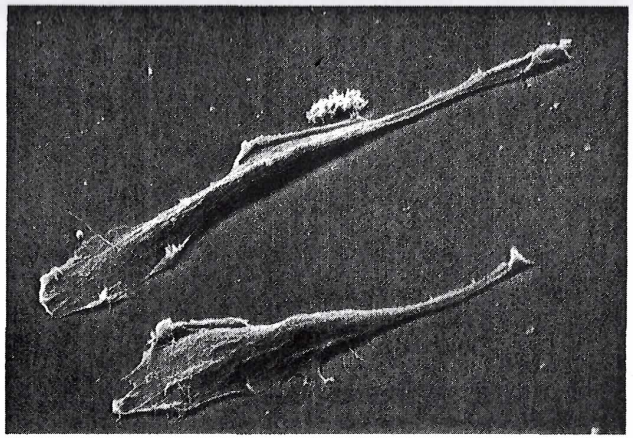

Figure 7.3. Fibroblast migration on a surface: many tissue-derived cells, such as the fibroblast shown here, migrate after attachment to a solid surface. The cells have a noticeable polarity, with a broad leading edge that faces the current direction of migration. (Photograph courtesy of Guenter Albrecht-Buehler.)

in a cyclic fashion, with a peak velocity of 50–60 nm/s ($\sim 3\,\mu$m/min) and an average net speed of 5.5 nm/s ($\sim 0.3\,\mu$m/min) [44], which is approximately equal to the speed of forward motion for the whole cell (Table 7.2). Other edges of the cell are relatively stationary. The leading edge is considerably thinner (in the direction normal to the substrate) and stiffer than other edges of the cell. This membrane activity is probably related to actin polymerization and actin fiber rearrangement within the peripheral membrane. In the region of active movement, the membrane sweeps over an area of the substrate; during this process, cell adhesion molecules in the membrane have an opportunity to bind to counterligands on the substrate. If binding occurs, a new site of adhesion may be formed and subsequently stabilized by the participation of additional receptor–ligand binding pairs.

Cells crawl across a substrate through a repetitive process of lamellipodial searching, adhesion at a new surface site, and detachment from an old site. As the leading edge of the membrane moves forward, the cell stretches because it is constrained by previous adhesion sites (Figure 7.5). Tension in the cell increases, due to this slow stretching and to active contractions of cytoskeletal elements in the cell. Eventually, if the cell is to move forward, the adhesions at the trailing edge of the cell must release, causing the stretched cell to relax with an overall advance in the location of the cell center-of-mass.

This mechanistic model of cell migration predicts that cell migration speed should be optimal at intermediate substrate adhesiveness [45]. When adhesion is too weak, new attachment sites cannot form at the leading edge; when

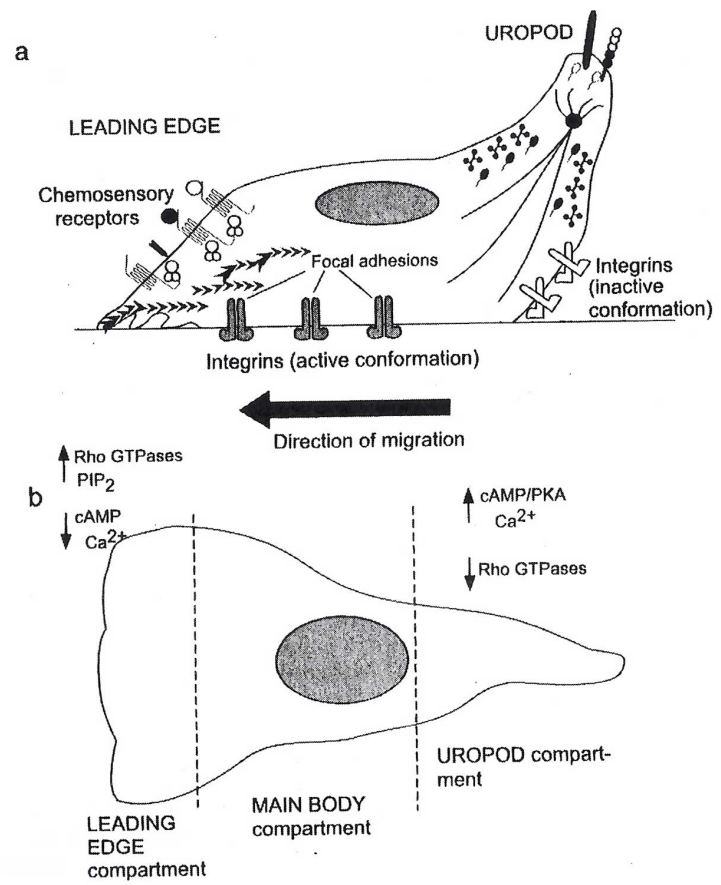


Figure 7.4. Mechanisms for development of polarity in leukocytes (adapted from [43]). The polarized morphology of leukocytes results in the formation of distinct front and rear regions with different biochemical compositions, membrane compositions, and functions. (a) Side view, indicating the distribution of cell surface molecules and cytoskeletal elements. (b) Top view, detailing the biochemical compartments within a migrating cell.

adhesion is too strong, old attachments sites cannot release at the rear. This prediction has been confirmed experimentally (Figure 7.6). Molecular biological tools and engineering analysis allow quantification of these mechanisms. Using cells with genetically engineered integrin receptors, the speed of cell migration was shown to depend on the number of receptor–ligand bonds between the cell and the substrate (which depends on both receptor number





Table 7.2
Typical Cell Speeds and Persistence Times

| Cell Type | $P$ (min) | $S$ (μm/min) | $\mu$ (cm$^2$/s) |
|---|---|---|---|
| Neutrophils | 1–4 | 20 | $30 \times 10^{-9}$ |
| Macrophages | 30 | 2 | $10 \times 10^{-9}$ |
| Fibroblasts | 60 | 0.5 | $1.2 \times 10^{-9}$ |
| Endothelial cells | 300 | 0.4 | |
| Smooth muscle cells | 240–300 | 0.5 | $6.2 \times 10^{-9}$ |
| Neurons on laminin [74] | | 1–3 | |
| Cerebellar granule cell neurons migrating on astroglial fibers [75] | Saltatory | ~ 1 (with pauses and long breaks) 0.05 – 0.3 (when observed over several hours) | |
| Cerebellar granule cell neurons migrating on laminin-coated or astroglial membrane-coated glass fibers [76] | Saltatory | ~ 0.1 | |

The variables are defined in the text: $P$ is the persistance time, $S$ is the speed of migration, and $\mu$ is the random motility coefficient.

in the cell and ligand density on the surface) and the affinity of each bond [46]. These experimental results confirm the predictions of a mathematical model that relates cell motile speed to the strength of cell–substrate adhesion (Figure 7.7). The model also predicts the influence of rheology and intracellular force generation on motile speed.

### 7.2.3 Patterns of Migration in Cell Populations

The motion of individual cells in culture can be tracked by time-lapse optical microscopy. When observed over a sufficiently long time in a uniform environment (that is, one without spatial gradients in properties of the fluid or solid phase), the migratory path of each cell appears to be random and bears a striking similarity to the random paths of particles in Brownian motion (Figure 7.8). Characterization of the speed of cell migration is therefore often performed by analogy with the methods for characterizing the speed of particles in Brownian motion, which is reviewed and applied in the next subsections.

**Random Migration.** A solute particle in Brownian motion moves as a result of collisions with other particles that constitute the solvent phase; momentum is transferred to the Brownian particle by each collision. A particle, therefore, moves with a velocity $\overline{V}$ until another collision causes it to change directions and move with new velocity $\overline{V}'$. Changes in direction are instantaneous and

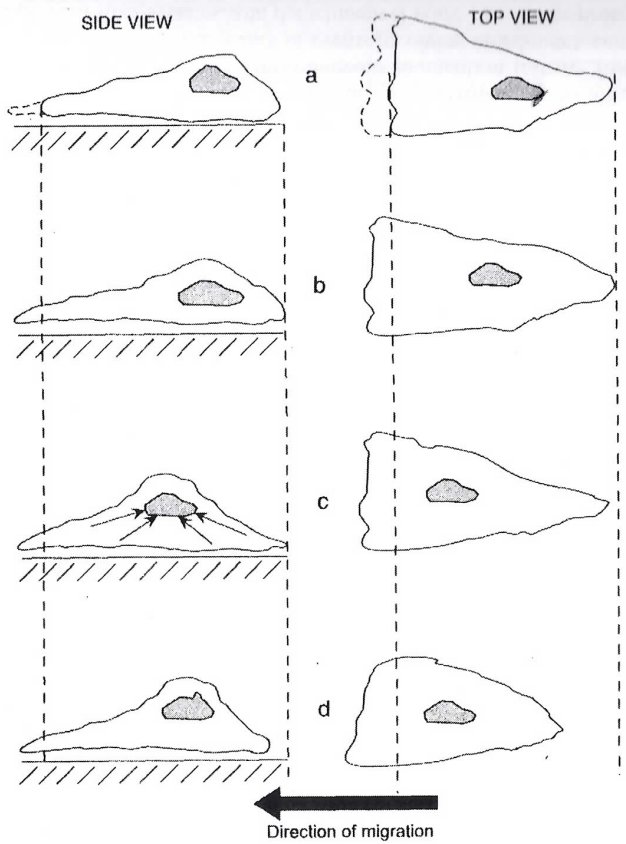


Figure 7.5. Mechanism of cell migration along a flat surface. For surface-attached cells, such as fibroblasts, migration occurs by a sequence of events: (a) protrusion of the lamellipodium at the forward edge of the cell; (b) formation of new adhesion sites at the forward edge; (c) accumulation of tension within the cell; and (d) release of adhesion sites at the trailing edge. Compare this schematic diagram to the photograph of the cells in Figure 7.3.

random, since the collisions occur from all directions with equal probability. The time between directional changes, or the time between collisions, depends on the density of molecules in the fluid. The average time between collisions can be estimated from the average distance the particle travels between collisions (called the mean free path) and the average speed $|\overline{V}|$. For oxygen at standard temperature and pressure, the mean free path is $1.6 \times 10^{-5}$ cm (1600 Å) and the average time between collisions is $4 \times 10^{-10}$ s [47] (p. 422).





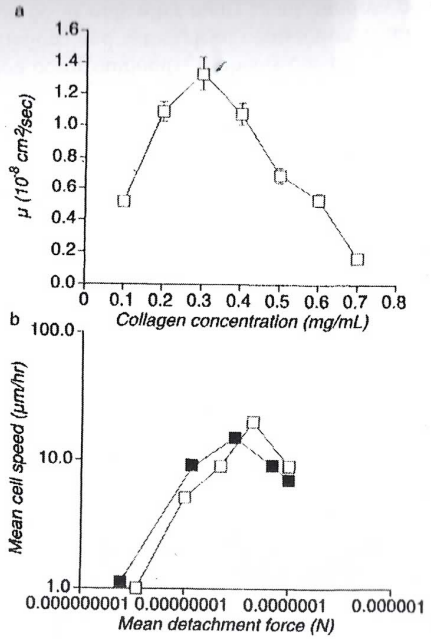


Figure 7.6. Cell motile speed has an optimum. The speed of random migration was measured for neutrophils migrating through a three-dimensional collagen gel (a) and for CHO cells migrating along a fibronectin-coated surface (b). Experimental results are redrawn from [56] and [46].

In a liquid, the higher particle density makes these times and distances even smaller. Therefore, the movement of a collection of Brownian particles is typically characterized by the rate of increase in the mean-squared displacement over observation times that are very long with respect to the mean free path interval. As described in the appendix section of this chapter, this behavior can in general be completely characterized by a diffusion coefficient $D$, which accounts for dispersion of the particle population that results from this process of repeated collision and random directional change.

Migratory paths of cells are similar to those of particles in Brownian motion. But do they result from the same underlying mechanism? The Stokes–Einstein equation provides a quantitative relationship between the rate of dispersion due to thermal fluctuations (that is, the diffusion coefficient $D$) and drag due to viscosity on the diffusing particle [48]:

$$D = \frac{k_B T}{6\pi\eta a} \tag{7-1}$$

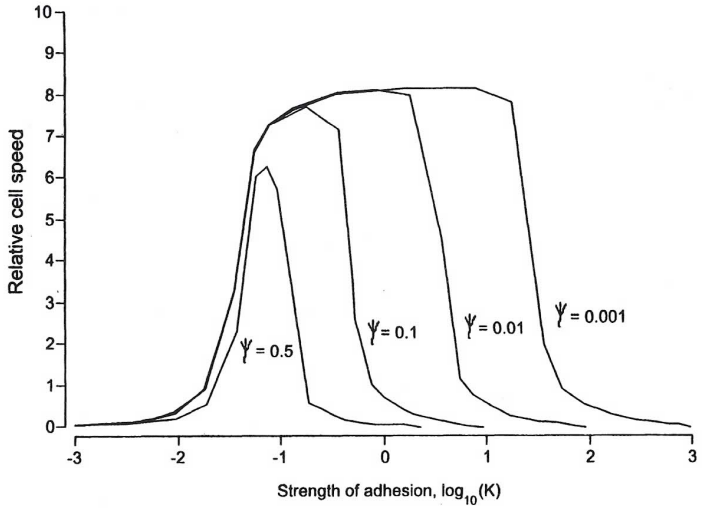


Figure 7.7. The characteristics of cell migration agree with mathematical models such as the model predictions from Figure 4 of [45]. The variable $K$ is related to the strength of cell–substrate adhesion and $\psi$ is a property of the cell population which depends on the rheological properties of the cell and the efficiency of force generation (see [45] for details).

where $k_B$ is Boltzmann's constant, $T$ is absolute temperature, $a$ is the particle radius, and $\eta$ is the viscosity of the fluid. This expression can be used to estimate the diffusion coefficient for particles of different sizes (Table 7.3). Neutrophils migrate through a collagen gel $\sim 20$ times faster than you would expect them to diffuse in water, in spite of the fact that the collagen gel is considerably more viscous than water. This rapid motility requires metabolic activity from the cell; dead or fixed cells can still move as Brownian particles (with the diffusion coefficient predicted by Equation 7-1), but they cannot migrate through a gel or over a surface.

Although the migratory path of a cell shares many similarities with the motion of Brownian particles, there are also important differences. The most striking difference is the observation that cells move in the same direction—at nearly constant speed—when observed over short experimental time intervals. The duration of the interval varies with cell type, but is generally on the order of one minute for rapidly moving cells such as neutrophils. This difference is sensible; cells do not move by instantaneous momentum transfer from other particles, but rather by a sequence of events involving attachment, contraction of intracellular fiber systems, and detachment (Figure 7.6). These processes are not instantaneous but require extensive rearrangement of internal cell structures as well as binding reactions between the cell surface and the substratum.





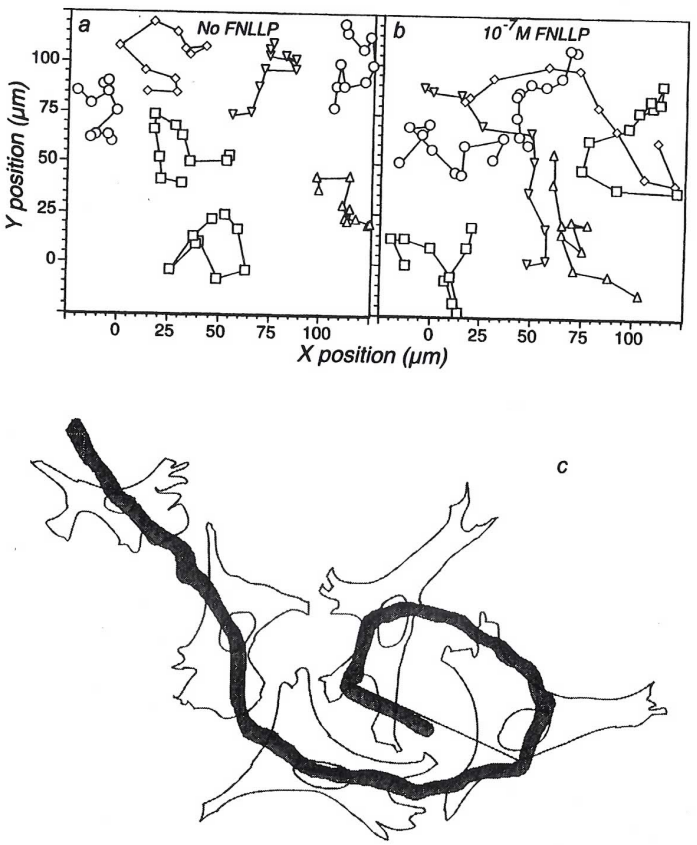


Figure 7.8. Pattern of migration for motile cells. This figure illustrates the migration of cells attached to surfaces and cells suspended in gels. In panels (a) and (b), each symbol represents a neutrophil that is migrating through a collagen gel. The position of the symbol represents the $(x, y)$ position of the cell at a specific point in time; intervals of 1.5 min separate each symbol along the connected paths. The cell population was either not exposed (a) or exposed (b) to an agent that leads to faster migration. In panel (c), the characteristic changes in cell shape, as well as the position of the cell center of mass, are shown for a fibroblast migrating on a tissue culture surface (redrawn from [71]).

Changes in direction occur randomly, probably due to the stochastic nature of the membrane adhesions and cytoskeletal contraction events, but substantial changes in direction occur only after many small, random perturbations have accumulated. The migratory path looks like Brownian motion only if it is viewed as a series of "snapshots" of particle position taken at suitably long

Table 7.3
Diffusion Coefficients for Particles Estimated from Stokes–Einstein Equation

| | Radius (μm) | $D$ ($10^{-7}$ cm$^2$/s) |
|---|---|---|
| *Particle diffusion* | | |
| $O_2$ in water | 0.00018 | 200 |
| Protein in water | 0.003 | 5 |
| Neutrophil in water | 5 | 0.005 |
| *Migration* | | |
| Neutrophil in 3D collagen gel | 5 | 0.1 |
| Fibroblast on glass surface | 5–10 | 0.01 |
| Pigmented retinal cell in neural aggregate | ~5 | 0.00005 |

See also [56, 73].

intervals (as shown in Figure 7.8). Higher resolution observations reveal steady forward motion and continuous directional changes; for many cells, the physical part of the cell at the leading edge remains constant, even as the cell turns. These observations suggest that cell migration occurs by a different physical process than the collision–run–collision process that yields Brownian motion.

In spite of these fundamental differences, studies of cell migration profit by comparison of cell movement with the statistical process of Brownian motion. Therefore, the physics of Brownian motion—or the statistics of a random walk process—are introduced in the next section (and a short background section is included at the end of this chapter).

**Directed Migration.** Cells respond to their environment; rates of migration depend on the composition of the local environment. Receptors on cell membranes bind to soluble factors (such as hormones, nutrients, and growth factors) and surface-bound factors (such as the ECM molecules reviewed in Chapter 6). If the environment contains uniform concentrations of diffusible and immobilized chemicals, cell migration is usually random. However, if spatial variations are present, cells are capable of detecting these gradients and changing their migration behavior. This process of moving in response to gradients is called taxis and is critical to the regulation of almost every biological event that involves cell movement.

A variety of cell responses can lead to taxis: enhanced turning towards the stimulus (topotaxis), increased cell speed when the cell is oriented towards the stimulus (orthotaxis), or decreased turning when the cell is oriented towards the stimulus (klinotaxis). Cell movement is influenced by spatial gradients of dissolved chemicals (chemotaxis), cell adhesion (haptotaxis), light (phototaxis), and other signals. Certain agents cause chemokinesis, a change in the kinetics of cell random migration that does not require a gradient but depends on the total concentration of an agent in the environment; chemokinesis is further classified into orthokinesis (due to variations in cell speed) and klinokinesis (due to variations in cell persistence time). Chemokinetic changes can lead to overall cell accumulation at a stimulus site; for example, an agent that



decreases cell speed will cause cells to accumulate at the site of maximum concentration (since cells that arrive at that spot are unable to move away).

## 7.3 Quantitative Methods for Describing the Movement of Individual Cells

### 7.3.1 Random Migration of Cells

The pattern of random migration of cells on a surface differs from Brownian motion: cell motions are usually observed at time intervals that are less than the time required for the cell to make a significant directional change. Observation times are relatively much longer than for particles in Brownian motion; the time between direction changes for albumin is $\tau = 4 \times 10^{-12}$ s (see Appendix of this Chapter), much smaller than the times relevant for cell movement. Cell migration is an example of a persistent random walk; at short observation times the cell appears to persist in whatever direction it is currently migrating, but at long observation times the migration is random (that is, similar to Brownian motion). If migrating cells were behaving as particles in a random walk, the mean squared displacement would increase linearly with time (as shown in Equation 7A-12 at the end of the chapter):

$$\langle d^2(t) \rangle = 4\mu t \tag{7-2}$$

where $\mu$ is the random motility coefficient for the cell population and $d_i(t)$ is the displacement of an individual cell at time $t$: $\bar{d}_i(t) = \bar{r}_i(t) - \bar{r}_i(0)$ with $\bar{r}_i$ the position of the cell in some appropriate coordinate system.

Equation 7-2 is written to describe the random motion of cells on a two-dimensional surface. A persistent random walk can be described by a modified form of Equation 7-2, which accounts for the persistence in directional migration observed at short times:

$$\langle d^2(t) \rangle = 4\mu(t - P + Pe^{-t/P}) \tag{7-3}$$

where $P$ is the persistence time, which is related to the time between significant changes in direction for a migrating cell (Figure 7.8c). These two relationships (Equations 7-2 and 7-3) are shown in Figure 7.9.

The persistent random walk model was first applied to the migration of fibroblasts on a tissue culture surface [22]. The dynamics of Brownian motion were simulated by a simple random walk model in which particles change position suddenly and randomly; Brownian motion is more correctly described by considering fluctuations that arise in particle velocity instead of position [49]. The persistent random walk model can be developed from the Langevin equation (see [50] for a more complete description of this equation and its use in modeling Brownian particles):

$$dv(t) = -\beta v(t)dt + dW(t) \tag{7-4}$$

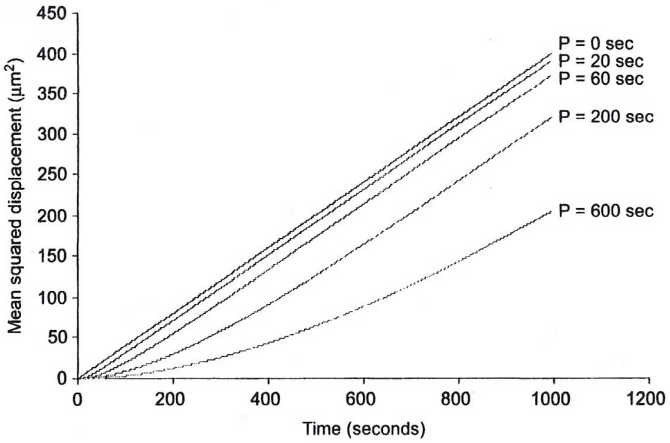


Figure 7.9. Mean-squared displacement for persistent random walks. The figure shows several plots of mean-squared displacement versus time, calculated from Equation 7-3, with the persistence time increasing over the range 0 to 600 s.



where $v(t)$ is the cell velocity and $W(t)$ is the stochastic Weiner process (that is, Gaussian random noise with a mean value of zero and a variance $\alpha dt$). This differential equation specifies the two forces that contribute to changes in cell speed: cell speed is decreased by drag, a deterministic force that is proportional to cell speed with a characteristic coefficient $\beta$, and cell speed is influenced by a stochastic driving force that is characterized by the Weiner process. For one-dimensional movement of a population of cells whose velocities are described by this stochastic differential equation, it is possible to demonstrate that the mean squared displacement and mean squared speed of the population are [51]:

$$\langle d^2(t)\rangle^{1D} = \frac{\alpha}{\beta^3}(\beta t - 1 + e^{-\beta t}) \tag{7-5}$$

$$\langle S^2\rangle^{1D} = \frac{\alpha}{2\beta} \tag{7-6}$$

where $d$ is the displacement of a cell after time $t$ and $S$ is the cell velocity, $v(t)$. As before, the bracketed terms on the left-hand sides of Equations 7-5 and 7-6 indicate mean squared quantities for the cell population. This description can be extended to higher dimensions by using the Pythagorean theorem:

$$\langle d^2(t)\rangle^{2D} = \langle d^2(t)\rangle^{1D} + \langle d^2(t)\rangle^{1D} = 2\frac{\alpha}{\beta^3}(\beta t - 1 + e^{-\beta t}) \tag{7-7}$$

$$\langle d^2(t)\rangle^{3D} = 3\frac{\alpha}{\beta^3}(\beta t - 1 + e^{-\beta t}) \tag{7-8}$$



$$\langle S^2\rangle^{2D} = \langle S^2\rangle^{1D} + \langle S^2\rangle^{1D} = \frac{\alpha}{\beta} \tag{7-9}$$

$$\langle S^2\rangle^{3D} = \frac{3\alpha}{2\beta} \tag{7-10}$$

It is more convenient to replace $\alpha$ and $\beta$, which describe the variance of statistical fluctuations and resistance to cell motility, respectively, by other terms that are related to more easily measured phenomena: that is, the cell speed, $S_n$ = root mean square speed for $n$-dimensional migration, from Equations 7-6, 7-9, and 7-10:

$$S_n = \sqrt{\langle S^2\rangle^{nD}} = \sqrt{\frac{n\alpha}{2\beta}} \tag{7-11}$$

and the persistence time:

$$P = \frac{1}{\beta} \tag{7-12}$$

With these terms defined, Equations 7-5, 7-7, and 7-8 can be written in the more general form:

$$\langle d^2(t)\rangle^{nD} = 2(S_n)^2 P\left(t - P + Pe^{-t/P}\right) \tag{7-13}$$

Finally, the random motility coefficient is defined from Equation 7-13:

$$\mu = \frac{\alpha}{2\beta^2} = \frac{(S_n)^2 P}{n} \tag{7-14}$$

so that the expressions for mean square displacement become

$$\langle d^2(t)\rangle^{nD} = 2n\mu(t - P + Pe^{-t/P}) \tag{7-15}$$

When $t \gg P$, Equation 7-15 reduces to 7-2 (and analogous equations for one- and three-dimensional random walks). For time scales that are large compared to the persistence time of a cell, the random migration of cells in a uniform environment can be described using the mathematical methods developed for molecular diffusion, which are reviewed in a number of texts [52–54].

This stochastic differential equation, Equation 7-4, can also be used as the basis of computer simulations of cell migration [55]. Using this approach, the pattern of migration for a small population of cells with the desired speed and persistence ($S$ and $P$) can be simulated. This method is useful for visualizing the influence of cell motility parameters ($S$, $\mu$, and $P$) on patterns of migration (Figure 7.10). It can also be used to test statistical methods for estimating motility parameters from experimental data [56].

The persistent random walk model has been used to characterize the rate of migration for a variety of cells; some examples are listed in Table 7.2. In general, the persistence time increases as the cell speed decreases. The average distance traveled between directional changes is approximately $S \times P$, or the mean free path length. The observation that $S$ and $P$ are reciprocally related is

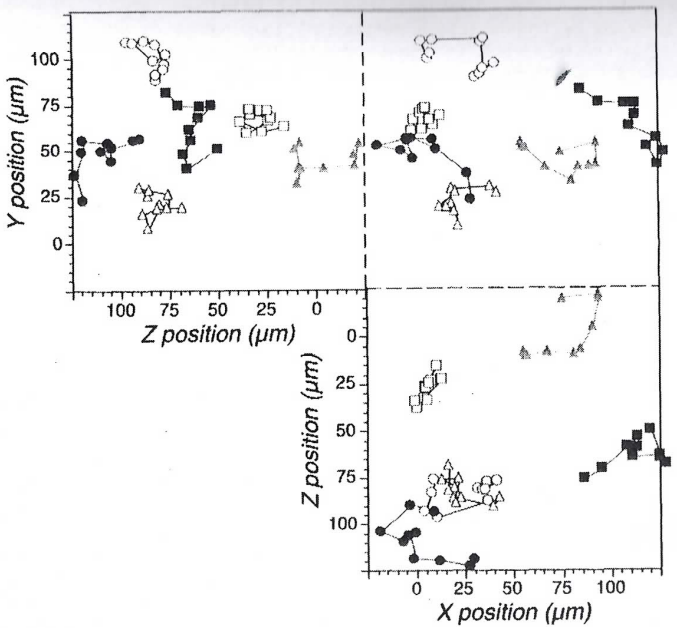


Figure 7.10. Migration paths for cells simulated using the stochastic differential equation. See text for details on the simulation procedure (more details are provided in [45]). This diagram shows several individual cells (each cell represented by a different symbol) migrating in a three-dimensional space.

reasonable, since the mean free path length must be optimal for cells to move productively: if $S \times P$ were too low, the cell would get nowhere, and if $S \times P$ were too high, the cell would move too far in the wrong direction.

### 7.3.2 Chemotaxis

Chemotaxis is the directed migration of cells in response to a gradient of a chemical signaling agent, or chemoattractant. Since migrating cells have a polarized morphology (that is, the front and rear of a migrating cell can be distinguished), a simple method for assessing the strength of a chemoattractant is to observe the percentage of cells that are migrating in the "correct" direction; usually, the "correct" direction is up the gradient, towards higher concentrations of the agent. The strength of a chemoattractant depends on the absolute concentration of the attractant and the steepness of the gradient (Figure 7.11). In general, the activity of a chemoattractant increases with concentration up to some optimal value, above





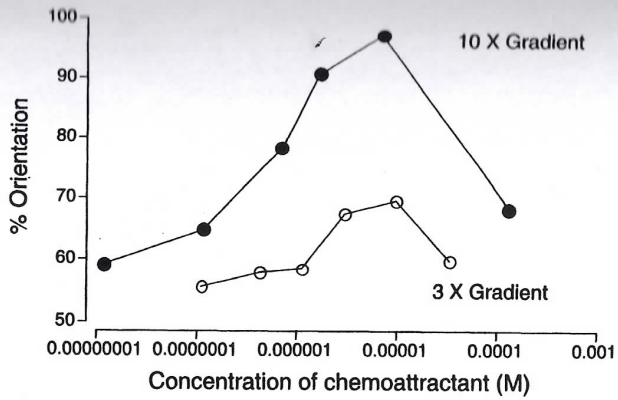


Figure 7.11. Orientation of neutrophils in a gradient of the peptide fMMM (redrawn from [23]). For a gradient applied in one dimension, as in this example, 50% of randomly migrating cells will be moving in the "correct" direction.

which the activity decreases; for formylated peptides, the concentration of optimal activity is $\sim 10^{-6}$ to $10^{-5}$ M.

The chemotactic movement of a population of cells can be characterized by the chemotactic index, CI:

$$\mathrm{CI} = \frac{\langle d \rangle}{L_{\mathrm{path}}} \left\{ 1 - \frac{P(1 - e^{-t/P})}{t} \right\}^{-1} \tag{7-16}$$

where $\langle d \rangle$ is the straight-line distance traveled towards the attractant source and $L_{\mathrm{path}}$ is the total distance traveled. For observation times that are large compared to the persistence time, that is, $t \gg P$, this expression reduces to:

$$\mathrm{CI} = \frac{\langle d \rangle}{L_{\mathrm{path}}} \tag{7-17}$$

The chemotactic index is equal to zero for perfectly random migration and one for perfectly directed migration. Computer simulations, similar to the ones described earlier for cells moving in persistent random walks but with a selected bias added to either the speed or direction of migration, have been used to study patterns of random migration in the presence of chemoattractants. These simulations have yielded insight into the mechanisms of signaling during directed migration [55, 57].

How do cells recognize the presence of chemoattractants? What mechanism does a cell use to determine the direction of a chemical gradient? These questions are not fully answered [58], but are best understood in bacterial cells, amoebae, and leukocytes from mammals. A bacterium swims by coordinated rotation of helical protein complexes called flagella [59]. Flagellar rotation is

generated by a motor which connects each flagellum to the cell wall; energy for rotation is provided by a proton flux across the plasma membrane. Since flagella are helical, clockwise and counter-clockwise rotation can produce different outcomes. In *E. coli*, when all of the flagella (each one is a left-handed helix) are rotating counter-clockwise, the cell is propelled smoothly forward through the fluid medium with all the flagella moving as a coordinated unit (Figure 7.12a). During clockwise rotation, the individual flagella pull the cell body in different directions, leading to rapid changes in cell direction; the cell appears to be tumbling (Figure 7.12b). The direction of flagellar rotation changes during a period of cell migration; when observed over time, the cell alternates between smooth swimming (running) and tumbling. At the end of a tumble, the cell is most likely to be pointed in a different direction than it was prior to the tumble. Chemoattractant chemicals act by modifying the frequency of clockwise rotation or tumbling; when a cell experiences increasing chemoattractant concentration (that is, when it is swimming up a chemical gradient), the frequency of tumbling decreases. Chemoattractants exert their effects by binding to receptors in the periplasmic space (Table 7.4); receptor binding activates an internal signal, involving a cascade of phosphorylation of proteins of the Che family (Figure 7.12).



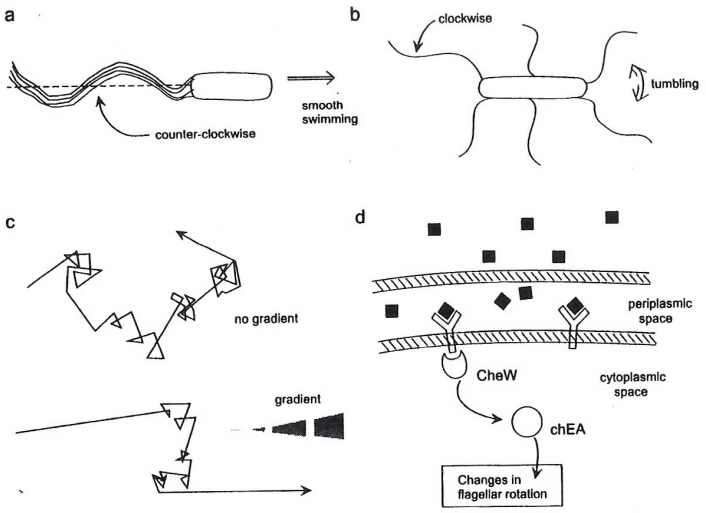


Figure 7.12. Mechanism of chemotaxis in *E. coli* bacterial cells (diagram adapted from Chapter 18 of [59]). The left-handed *E. coli* flagella produce (a) smooth forward swimming by counter-clockwise rotation, and (b) tumbling by clockwise rotation. (c) Chemoattractants modify the relative frequency of tumbles, producing net movement toward the source. (d) Chemoattractants act through a signal transduction pathway.



Table 7.4
Chemicals that Stimulate Directed Cell Movement

| Cell | Attractant | Receptor |
|---|---|---|
| *E. coli* | Serine | *Tsr* protein |
| | Aspartate | *Tar* protein |
| | Glucose | *Trg* protein |
| | Dipeptides | *Tap* protein |

Some of the chemicals that can attract bacterial cells are listed.

Bacterial cells detect the presence of chemical gradients by moving and sampling concentration at different places. This is a sensible strategy for small (1 μm), rapidly moving cells, since detection by another mechanism—such as comparing concentration at two different locations on the cell at the same moment in time—would be difficult. Amoebae and leukocytes, however, are substantially larger, so other mechanisms are possible. In fact, leukocytes appear to extend pseudopodia in the correct direction (that is, up the gradient) even before they initiate motion, suggesting that sensing is independent of motion (and time).

Chemotaxis in leukocytes and other eukaryotic cells is regulated by a G protein-linked receptor with seven membrane-spanning regions (Figure 7.13). Binding of a chemoattractant leads to a conformational change in the receptor, which activates the G protein by causing the binding of GTP and the release of GDP. The activated G protein stimulates activity of adenylyl cyclase (or another effector enzyme), causing generation of the second messenger cAMP. Cells move in the direction of highest concentration of chemoattractant, but the receptor protein and G protein β subunit remain uniformly distributed throughout the cell surface. The generated signal, which eventually leads to an accumulation of actin-binding proteins at the leading edge of the cell, must become spatially localized at some step in the signal transduction pathway between G protein activation and actin-binding protein accumulation. The most recent evidence suggests that binding of proteins with a pleckstrin homology (PH) domain to the G-protein β subunit is an important step in localization.