Chapter 11
Equation-Based Models of Wound Healing and Collective Cell Migration

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Introduction

Wound healing is a physiological process of repair of a tissue that has been structurally damaged. The most common wounds disrupt one of the epithelial tissues that protect the internal and external surfaces of the body and act as barriers against invasion by microorganisms. Such tissues include skin, corneal tissue, and the epithelial lining of the digestive tract (including the mouth and esophagus), respiratory tract (including the alveoli), urinary tract, and reproductive organs. Any disruption of these tissues can lead to serious health conditions or developmental abnormalities; for example, a wound in the gut epithelium can lead to necrotizing enterocolitis, which is the leading cause of death from gastrointestinal disease in preterm infants. Internal wounds may also arise due to physical overexertion or blunt force trauma. The biology of wound healing is reviewed in Chapter 10.

The ability to heal wounds is closely related to the regenerative ability of the organism to restore the function of many organs. Wound healing generally proceeds in four stages, although these stages differ in details depending on the location of the wound [1–4]. The **first stage** is hemostasis, characterized by the leakage of fluids out of broken blood and lymphatic vessels and the delivery of inflammatory cells and platelets to the wound. The platelets trigger vasoconstriction to reduce blood loss and form a blood clot to fill the wound. The clot contains fibrin molecules that provide an extracellular matrix (ECM) for the migration of leukocytes and fibroblasts, which are cells responsible for eliminating pathogens and repairing the

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tissue, respectively. The **second stage** (2–3 days for skin wounds) is an inflammatory reaction marked by neutrophil recruitment followed by macrophage infiltration. Neutrophils phagocytose necrotic tissue, kill bacteria that enter the wound, and produce chemoattractants to recruit macrophages. Macrophages secrete pro- and anti-inflammatory cytokines that regulate inflammation and trigger the phagocytosis of pathogens and cell debris. Macrophages also secrete growth factors necessary for the third stage of wound healing. The degree of inflammation that ensues is directly related to scar formation [5]; for instance, the lack of inflammation in embryos leads to scarless wound healing. The **third stage** of the process (3–10 days for skin wounds) is the recovery of the tissue via cell migration and proliferation. The wound is also infiltrated by fibroblasts, which initiate the formation of the collagen matrix to provide mechanical strength for the disrupted tissue and keratinocytes to regulate the reepithelialization process. In addition, new capillaries are grown by extension and sprouting in a process called angiogenesis, and the development of acute granulation tissue is initiated. This transitional granulation tissue replaces the provisional wound matrix and is characterized by a high density of fibroblasts, granulocytes, and macrophages. The **fourth stage** of the wound healing process is tissue remodeling, which can take anywhere from 21 days to 1 year for skin wounds. During this process, the formation of granulation tissue ceases, and collagen III, which forms a basket weave-like structure in the ECM, is replaced by collagen I, which is stronger and oriented in parallel bundles. Furthermore, the wound contracts and decreases the surface of the developing scar.

These four stages have been observed, measured, and assessed in a wide range of experimental and clinical scenarios. In some cases, shallow wounds are studied using in vitro experiments known as scratch-wound assays (depicted in Fig. 11.1), which are designed to track the migration and proliferation of a monolayer of cells. In a scratch-wound assay, cells are cultured (typically on a glass coverslip), grown to confluence, and then scraped with the tip of a pipette to create a gap that represents a wound in the tissue layer. Medium is continuously perfused across the cells, and the motion and deformation of cells in the layer is analyzed. Other in vitro assays include three-dimensional organ explant cultures or three-dimensional sprouting invasion assays from mesenchymal cells overlaid onto a three-dimensional ECM or implanted as a multicellular spheroid [6]. Using such assays, numerous studies have evaluated how extracellular stimuli, geometric anisotropy of substrates, or intracellular processes activate cell migration and trigger cell proliferation [7–11]. Measurements of the physical forces driving cell migration indicate that traction forces arise many cell rows behind the leading wound edge and extend across large distances [9]. Trepat et al. [9] demonstrated that individual cells at both the leading wound edge and inside the sheet engage in a “tug-of-war” that integrates local force generation into a global state of tensile stress. Mechanical forces exerted by epithelial cells were measured by du Roure et al. [11] using a technique that combines microfabrication of flexible substrates and multiple-particle tracking microscopy. Because each micropillar deflection is independent of its neighbors, the measured traction forces under the cells are uniquely determined.

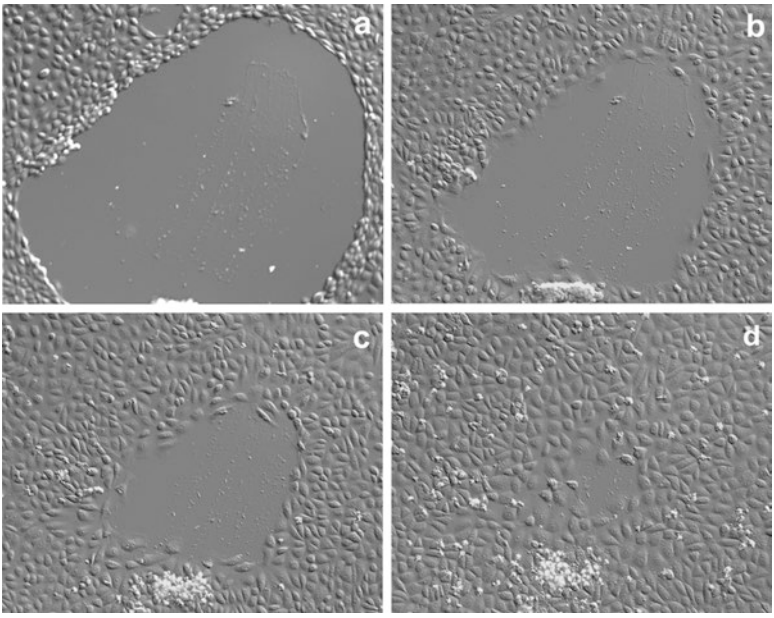

Fig. 11.1 Scratch-wound assay of intestinal epithelial cells. Large space void of cells denotes wound; surrounding region is the epithelial layer, which remains connected during closure. Panels **a**–**d** show the progression of wound closure after 0, 125, 250, and 500 min, respectively

In clinical settings, the progression of wounds, such as skin wounds or diabetic foot ulcers, are quantified according to measures such as the absolute wound area remaining, percentage of initial wound area remaining, wound volume remaining, or wound perimeter remaining. Some of these measurements can be difficult to obtain given the location of the wound or type of data needed, and, as a result, currently there is no universally accepted measure of wound healing [12]. In addition, although wound area is an obvious measure of wound closure, predicting healing time based on the percent of wound area healed tends to bias small wounds, and predicting healing time based on the absolute wound area healed tends to bias large wounds [13]. A reliable measure of wound healing time is of particular interest to both physicians and patients in order to determine effective treatment methods for various wounds.

Ultimately, a wound is considered healed once tissue functionality has been fully restored via the migration of cells into the wounded region and the proliferation of new cells to restore the original density of the tissue. Observations from multiple wound scenarios have shown that cell migration and proliferation as well as the overall healing time for a wound are affected by factors such as wound geometry, tissue type, cell–cell interactions, and the stage of the healing process (epithelialization, contraction, or proliferation). Combining these observations with mathematical modeling techniques may help to unravel the key aspects governing wound healing.

Modeling

As in the setting of sepsis, trauma, and other acute inflammatory conditions (see Chapter 2), mathematical modeling of wound healing is used to aid in the understanding of the complex processes involved in wound healing by providing a platform for testing various hypotheses regarding the interaction of wound healing components. Equation-based models describe biological processes by formulating interactions of individual biological components using a system of differential equations for variables that measure the concentrations of cells and chemicals in time and space. The equations are constructed using knowledge deduced from experiments and are calibrated using experimental data (for example, data could be used that describes the dependency of cell migration speed on the concentration of a chemoattractant). The equations are solved using a variety of analytical and numerical techniques and are used to predict the dynamics of cell populations within a wound. There are two classes of equation-based models in common practice—models based on ordinary differential equations (ODEs) and models based on partial differential equations (PDEs). The main difference is that ODEs can describe the time dependence of the wound healing process but not its spatial variability, while PDEs can describe both. In addition, there are also stochastic models that can include fluctuations in chemical concentrations and other random effects [14, 15]. A summary of these different types of models, many of which are described in more detail in this chapter, is provided in Table 11.1.

Equation-based models are beneficial in situations in which it is reasonable to assume that the components of the system are either of the same kind and respond identically to stimuli or are of different types, but their response depends only on the number of components at a given spatial location at a given time. For example, when fibroblasts are rebuilding the collagen matrix, the speed of rebuilding depends on how many fibroblasts are present, but not on where precisely each fibroblast is and how it moves about. In such a case, one may invoke the *continuum hypothesis* and assume that there is a density function $f(x, t)$ that depends on space and time, which describes the mass (or molar) density of the cellular component (cell or a chemical). The density can be understood in a statistical or probabilistic sense. In the first case, the quantity f is equal to the average number of components per unit volume centered at position x and measured at time t for a series of identical experiments; in the second case, f represents the probability of finding a component in that volume at time t. The use of the continuum hypothesis implies that we can only predict the behavior (motion and state changes) of population averaged properties, not of individual molecules or cells. In contrast to ODE models, models satisfying the continuum hypothesis preserve the possibility of properties varying in space.

By choosing a continuum formulation, we substantially reduce the amount of information needed to specify the state of the system. Imagine a 1-cm wound with 10^8 fibroblasts. In order to specify the fibroblasts' positions in 3-space, we would need 3×10^8 numbers. However, to define their spatial distribution, it may suffice to use a grid of spacing $100 \times 100 \times 100$ nodes (10 nodes per mm), which results in 10^6 values for the density and a 300-fold reduction in the amount of data needed to characterize the system. A coarser grid would result in even higher simplification

Table 11.1 Summary of equation-based and agent-based wound healing models

Model	Type	Examples	Phenomenon modeled	Tissue type
ODE		Cukjati et al. [20]	Wound area healing	Endothelium, etc.
		Johnson [17]	Wound area healing	Arteries, veins
		Bardsley et al. [5]	Wound area healing	General
		Baker et al. [18]	Wound area healing	Diabetic ulcers
		Jercinovic et al. [19]	Wound area healing	Pressure ulcers
		Menke et al. [21]	Fibroblasts; oxygenation	Dermis
PDE	Reaction–diffusion	Maini et al. [24, 25]	Migration; proliferation	Peritoneal
		Sherratt et al. [26, 27]	Migration; proliferation	Epidermis
		Sheardown et al. [29]	Migration; proliferation	Cornea
		Dale et al. [30]	Migration; proliferation	Cornea
		Tremel et al. [31]	Migration; proliferation	Fibroblast cells
		Gaffney et al. [33]	Migration; proliferation	Cornea
		Chen et al. [34]	Migration analysis	Tumor
		Dale et al. [36]	Migration analysis	Scar tissue
		Wearing et al. [37]	Migration; proliferation	Dermis
		Javierre et al. [4]	Migration; proliferation	General
	Continuum mechanical	Vitorino et al. [51]	Migration; proliferation	Endothelium
		Lee et al. [56]	Migration	Kidney cells
		Xue et al. [57]	Migration, oxygenation	Cutaneous
		Qi et al. [58]	Migration	Epithelium
		Arciero et al. [59]	Migration	Epithelium
	Cell signaling	Posta et al. [62]	MAPK activity	Epithelium
		Dale et al. [63, 64]	Fibroblasts; collagen	Scar tissue
		Murray et al. [66]	Wound contraction	General
		Palecek et al. [54]	Cytoskeleton-integrin	General
		Gaffney et al. [32]	Diffusion	Cornea
		Tranquillo et al. [67]	Migration; proliferation	Fibroblasts, ECM
		Olsen et al. [68]	Fibroblasts; proliferation	Scar tissue
		Sherratt et al. [69]	Wound contraction	Epithelium
		Murray et al. [70]	Morphogenesis	ECM
	Angiogenesis	Chaplain et al. [71]	New capillary formation	Tumor
		Anderson et al. [72]	New capillary formation	Cornea tumor
		Pettet et al. [75]	New capillary formation	Soft tissue
	Chemotaxis	Hillen et al. [77]	Chemical migration	General
		Schugart et al. [78]	Fibroblasts; oxygenation	Cutaneous
ABM		Dallon et al. [65]	Collagen deposition	Dermis
		DiMilla et al. [79]	Receptor-ligand binding	General
		Walker et al. [80, 81]	Migration; proliferation	Epithelium
		Bindschadler et al. [83]	Migration; proliferation	Scratch wound
		Ouaknin et al. [84]	Migration; chemotaxis	General
		Fozard et al. [86]	Collective cell migration	Epithelium
		Byrne et al. [87]	Cell expansion	General

and speedup in computation of the dynamical behavior of the system. Even more important is that we need not be concerned with the details of motion of individual cells; instead, the motion can be described in one of several standard ways (diffusion, chemotaxis, or convection) that we describe below.

The time rate of change of the density variable is expressed using an equation that involves partial derivatives of f with respect to the independent variables $\mathbf{x}$ and t. The most basic partial differential equation one can construct is the law of conservation of the number of cellular components in a given volume:

$$\frac{\partial f}{\partial t} = -\nabla \cdot \mathbf{J} + \sigma$$

Here $\mathbf{J} = (J_x, J_y, J_z)$ is a vector-valued variable that represents the flux of the component (migration or transport of the component away from the position $\mathbf{x}$), σ represents the local source or decay of the component, and $\nabla \cdot \mathbf{J} = \partial J_x / \partial x + \partial J_y / \partial y + \partial J_z / \partial z$ is the divergence of $\mathbf{J}$.

By using different relations between $\mathbf{J}$ and f, one can account for different types of transport such as (1) the *convection* of cells or molecules in the direction of velocity $\mathbf{v}$, defined by $\mathbf{J} = f\mathbf{v}$, and (2) the *diffusion* of cells or molecules, resulting from random motion of cells or molecules in all directions and defined by $\mathbf{J} = \nabla f$. A special case of convection is the *chemotaxis* of cells in which the direction of motion is defined by a gradient of a chemoattractant molecule with concentration c, i.e., $\mathbf{J} = f \nabla c$. The source σ is a function of f (expressing self-regulation of the component) and possibly other components (expressing the influence of components on each other). For example, in models describing the mechanics of a tissue, the variables are the mass density of the tissue, ρ, and the momentum of the tissue, ρ$\mathbf{v}$; the flux of the momentum is the stress tensor $\mathbf{T}$.

The system of PDEs formulated from known properties and interactions of cellular components forms the core of a PDE model. The remaining parts of the model are the definitions of the *domain* in which the equations are valid, the *initial values* of all variables across the domain, and the *boundary conditions* imposed at the boundary of the domain. The boundary conditions are typically defined as one of two types (1) Dirichlet conditions, representing a constant source or sink of the quantity and prescribed as a fixed value of the quantity at the boundary and (2) Neumann conditions, representing the flux of a quantity across the boundary and prescribed as the derivative of the quantity along the normal to the boundary.

The analysis of a PDE model starts with an examination of the existence and uniqueness of solutions. Contrary to strong results in the theory of ODEs, there are no generic results apart from the Cauchy–Kowalevski theorem guaranteeing local existence of a solution for a Cauchy problem. Global existence can be proved for a diffusion equation and systems of diffusion equations with the same diffusion constant. But in general, every system must be analyzed individually using techniques such as maximum principle, weak solutions, variational formulation, etc. (see, e.g., [16]). The next step in the analysis may be to search for special solutions that enable one to reduce the dimension of the equation. Such special solutions can be (1) a *steady state solution* in which f is independent of t, (2) a *self-similar solution*, which is invariant under a rescaling of the spatial and time variables, or (3) a *traveling wave solution*, which represents solutions invariant under the transformation $\mathbf{x} \to (\mathbf{x} - \mathbf{v}t)$. These special solutions are then analyzed for stability, i.e., invariance under a small perturbation. Stable solutions are of particular interest to various

applications since they describe the observed behavior of the system. If solutions do not converge to steady state solutions or traveling waves, they may approach singularities or blowups.

Once all information is extracted using analytical tools, numerical solutions of the system can be obtained. Numerical solutions of ODEs can be found using standard integration packages such as CVODE (available in C or FORTRAN) or MATLAB suite of integrators. A convenient free standalone program that allows the user to explore solutions of ODE systems is XPP. Numerical solutions of PDEs are obtained by converting the PDE system into a system of algebraic equations by transforming the derivatives into finite differences (finite difference methods) or by transforming the equations into a variational form formulated as integrals over appropriate test functions with simplicial support (finite element methods). User-friendly software packages have been developed that enable researchers with little knowledge of numerical mathematics to solve various types of PDE problems—see, e.g., the FEMLAB or Matlab Partial Differential Equation Toolbox.

ODE Models

The simplest ODE models of wound healing are phenomenological: they try to capture the time-dependent closure of the wound by formulating an equation for the wound area or circumference as a function of time and fitting the constants in the equation to observed data [5]. The majority of such models is based on linear or exponential functions that involve two parameters [17–19]. However, these are not sufficient to describe the initial delay of the healing process that is noticed in wound healing experiments. Cukjati et al. [20] formulated several ODE models by considering two, three, and four parameter functions of chronic wound healing and assessed their goodness of fit to 226 chronic wounds. They used a set of five criteria to qualitatively and quantitatively assess the model (statistical analysis of goodness of fit) and concluded that a delayed exponential model with three parameters is the most adequate for representing the healing process.

Mechanistic ODE models are based on formulation of equations describing the concentration of various components of the wound healing process. An example is the model of Menke et al. [21] which focuses on the second stage of the process (inflammation) by using an extension of an ODE model of inflammation [22, 23] to include tissue damage, pathogen level, inflammation, fibroblast concentration, and tissue oxygenation. The model is used to simulate impaired wound healing in hypoxic skin wounds with varied levels of contamination. Pathogen growth is assumed to depend on tissue oxygenation levels. The skin is assumed to have a baseline level of circulating fibroblasts, which increases in response to tissue damage and inflammation. The authors classify wounds as healed, nonhealing, or chronic and find parameter ranges for each type of outcome. Impaired wound healing is simulated in hypoxic wounds with varying levels of contamination, and the model is used to suggest possible components to target in therapies such as the fibroblast death rate and the rate of fibroblast recruitment.

PDE Models

PDE models of wound healing describe the spatial dependence of various components involved in the healing process and can predict the shape of the wound. Most existing PDE models focus on the third and fourth stages of wound healing process, i.e., on the repair of the epithelial layer and the remodeling of the scar tissue.

Reaction–Diffusion Models Repair of the epithelial layer is a combination of two processes migration of epithelial cells into the wound and cell proliferation. The simplest PDE model of wound closure that can be constructed is one that consists of a single equation for cell concentration with wound closure interpreted as a traveling wave of cell concentration. The equation commonly used in that interpretation is the Fisher–Kolmogorov equation, which is a reaction–diffusion equation with proliferation given by a logistic term. Maini et al. [24, 25] verified the validity of using the Fisher–Kolmogorov equation in a medical context by using a scratch-wound assay (for an example of a scratch-wound assay, see Fig. 11.1) and comparing model predictions with multiple experiments.

Both the migration and the proliferation of epithelial cells are regulated by growth factors. The first model to account for such chemical control was developed by Sherratt and Murray [26]. The model consists of two nonlinear reaction–diffusion equations that track epithelial cell density and a chemical regulating mitosis [the epidermal growth factor (EGF)] in the context of epidermal wound healing. The epidermis is assumed sufficiently thin and thus the tissue is modeled as two dimensional. The mitosis chemical is modeled as both an activator and inhibitor, and the effect of these two chemical behaviors on cell migration is investigated. The model was further analyzed in [27] by providing details for traveling wave solutions for cell density and chemical concentration. The results for wound radius as a function of time were shown to be consistent with experimental measurements. Clinical implications of the model were studied in [28], in particular the dependence of healing time on wound shape (e.g., cusped, ovate, and rectangular) and the dependence of predicted wound contours on the character of the growth factor.

The Sherratt–Murray model was extended to corneal epithelial wounds by Sheardown and Cheng [29] and by Dale et al. [30] who used the model to study the impact of increased mitotic and migratory activity due to an EGF. They also predicted the wound healing rate if the growth factor was applied only topically to the wound and found that the factors affecting migration include cell migration, cell-to-substrate adhesion, and cell mitosis. They noted that the model predicted a lag time immediately after wounding.

Tremel et al. [31] modified the Fisher–Kolmogorov equation to include the effects of cell density-dependent diffusion on wound healing. They assumed that diffusivity decreases with increasing cell density in order to capture contact-inhibited movement between cells so that cells slow, stop, or change direction when they encounter another cell in their path. In their study, cell tracking was performed on groups of cells in a wound healing experiment; in the images, it was observed that the cells initially located close to the wavefront traveled significantly greater

distances than cells starting farther behind the wavefront. Also, while the overall cell movement was directed, a significant amount of random motion was observed.

Several studies have modeled the influence of physiological electric fields on wound closure. In those studies, the PDE problem was formulated with a *free boundary*, i.e., a boundary whose position is changing in time. This change of position is governed by an additional equation. For example, Gaffney et al. [32] described the evolution of the free boundary problem for a system of two reaction–diffusion equations for cell density and chemical stimulus in the context of corneal wound healing. The formulation predicts a linear relation between the wound healing speed and the physiological electric field strengths over a physiologically large range of electric field strength. Spatial and temporal data on mitotic rates measured during corneal epithelial wound healing in a rat was studied by Gaffney et al. [33] who argued that earlier models were not adequate for the study of cell kinetics. Chen and Friedman [34] analyzed the Gaffney model [32] and applied a similar approach to predicting tumor growth [35].

In a subsequent paper, Dale et al. [36] presented a complex model for scar tissue formation in deep wounds and focused on the role of key chemicals in determining the quality of healing. The authors described wound healing as a traveling wave and investigated the factors controlling the speed of the wave. A more complex model accounting for the effect of the keratinocyte growth factor (KGF) was proposed by Wearing and Sherratt [37] who found that high KGF levels decreased the speed of healing but increased the cell division rate at a greater distance away from the wound edge. A comprehensive review of wound healing models of Sherratt and collaborators is given in [38] and [39].

Javierre et al. [4] also modeled the reepithelialization of the basal membrane of the epidermis by cell mitosis and migration in the presence of a generic EGF. The diffusion, depletion, and production of the concentration of the growth factor in the model are determined by a reaction–diffusion equation. The model assumes that cells become motile if the accumulated growth factor concentration exceeds a threshold value. A sigmoid function is used to relate cell mitosis and the growth factor concentration. Since cell migration is interrupted when the growth factor concentration drops below a threshold, cell motility is dose dependent in this model. Moreover, the wound closure rate is assumed proportional to the local curvature of the wound edge. Javierre et al. [4] analyzed the roles of diffusion, closure rate, and wound geometry on healing kinetics and concluded that healing is always initiated at regions with high curvatures, and that the evolution of the wound is sensitive to multiple physiological model parameters.

Continuum-Mechanical Models For the success of wound closure during the third stage of wound healing it is essential that the epithelial cells migrate collectively, in synchrony, so that the coverage of the wound is continuous without the formation of any holes in the remaining sheet. Cell migration at the single-cell level has been studied extensively over many decades [40–44]. In brief, each cell moves by a cyclic mechanism that proceeds through stages involving the formation of a lamellipodium, translocation of the nucleus in the direction of motion, and detachment of the

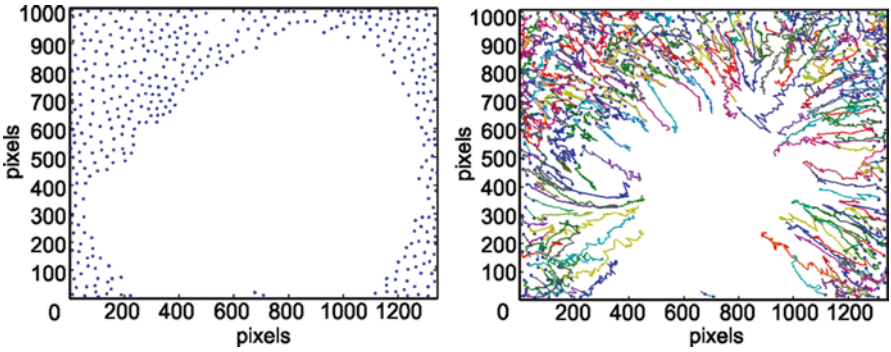

Fig. 11.2 *Left panel*: initial position of all cells in scratch-wound assay is indicated by *blue dots*. *Right panel*: cell trajectories of every initial cell position over the course of several hours

trailing edge [42, 45]. This mechanism is regulated by a complex signaling and regulatory network responsible for the underlying processes of actin polymerization and depolymerization, motor protein activation, and integrin formation and release.

Although the study of individual cell migration has been pursued vigorously, there is less understanding of the interactions that drive and synchronize collective cell migration in wound closure. Several mechanisms of closure have been proposed (1) a leader cell mechanism, (2) cooperative traction force mechanism, (3) steered migration mechanism, (4) differential adhesion hypothesis, and (5) differential interface tension hypothesis. In the leader cell mechanism [6], the cells at the edge of the wound are believed to change their phenotype and direct the migration of other cells towards the wound. In the cooperative traction force mechanism, cells near the edge of the layer exert coordinated forces that result in a cumulative stress within the layer and motion of cells towards the wound [9]. In the steered migration mechanism, the direction of autonomously migrating cells is changed in a gradual fashion by forces exerted on them by neighboring cells [46]. The differential adhesion [47] and differential interface tension [48] hypotheses stipulate that the cell layer evolves to minimize either the adhesion energy or surface tension of the constituent cells, which leads to the eventual wound closure.

As described earlier in this chapter, a typical experimental method used to study collective cell migration is the scratch-wound assay (Fig. 11.1). Farooqui and Fenteany [49] studied wound closure in Madin–Darby canine kidney (MDCK) epithelial cell layers and established that submarginal cells exhibit protrusive and migratory behavior similar to that of marginal cells. They found that the general direction of the coordinated cell movement was toward the center of the wound and the cell velocity within a sheet was found to be inversely proportional to the distance from the wound edge. Wound closure was shown to occur even if the motility of edge cells was inhibited, but it occurred at a slower rate [50]. Coordinated cell movement toward the center of a scratch-wound assay is depicted in Fig. 11.2. In the left panel, the starting positions of all cells of the scratch assay are denoted by

blue dots. In the right panel, colored lines define the trajectories of all the cells, with the blue dots indicating the starting point of the cell as in the left panel. The trajectories indicate the tendency of the cells to migrate towards the center of the wound.

Vitorino and Meyer [51] studied growth factor-induced migration of endothelial cell monolayers and proposed that the fibroblast growth factor (FGF) led to directed migration of leader cells but did not control cell migration and coordination of the follower cells. Mechanically robust and dynamic coupling of cells to one another and to the substrate is accomplished via adherens junction proteins, desmosomal proteins, and integrins [6, 52]. The cells in the interior are connected to the cells at the boundary by tight junctions, which prevent separation of the cells in the layer [53]. The level of adhesion between the cell and the substrate, moderated by integrins, was found to control the speed of wound closure [54]. The effects of substrate stiffness on cell traction forces were quantified for epithelial cells and fibroblasts, and it was shown that cell movement could be modulated by changing the stiffness of the substrate [8]. Trepat et al. [9] found that traction forces, applied by moving MDCK cells on the substrate, were smallest in the center of a cell colony and largest at the edge of the colony of cells moving radially outward. They estimated that tension in the cell layer increased with distance from the edge of the cell colony and argued that accumulated traction stresses were balanced by the forces within the cell sheet; the interplay of these two stresses was described using a tug-of-war model. In several studies, a release of tension was observed within the cell layer once a wound was induced [7, 55]. Block et al. [55] compared cell-sheet migration in wounds induced by different methods and hypothesized that the release of spatial constraints initiates a healing response. However, this hypothesis is difficult to verify experimentally since it is hard to eliminate all possible methods (such as biochemical communication) that may contribute to collective cell migration.

All the models described so far represented migrating cells using reaction–diffusion equations for cell density. Such equations are based on the diffusion mechanism for cell migration, which provides no guarantee of continuity of the cell layer. The process of collective cell migration is complex and requires fundamentally different, mechanics-based models. Lee and Wogelmuth [56] developed a model in which an MDCK cell layer was represented as a viscous liquid with orientation, similar to a liquid crystal; the layer orientation was equated with the direction in which the cell exerts a crawling force. They formulated equations of balance of forces on the cells and, using numerical solutions, were able to reproduce not only wound closure dynamics but also the irregular, undulating, progression of the edge of the layer typical for scratch-wound assays, without the need to specify leader cells. Xue et al. [57] developed a continuum model of ischemic dermal wounds with the wound boundary represented as a free boundary that moves with the velocity of the ECM at the wound edge. The model was used to predict how ischemic conditions may impair wound closure.

Mi et al. [58] recently developed a one-dimensional continuum mechanical model of a migrating IEC-6 enterocyte cell sheet to study the influence of lipopolysaccharide (a protein found in the coat of Gram-negative bacteria) and integrin

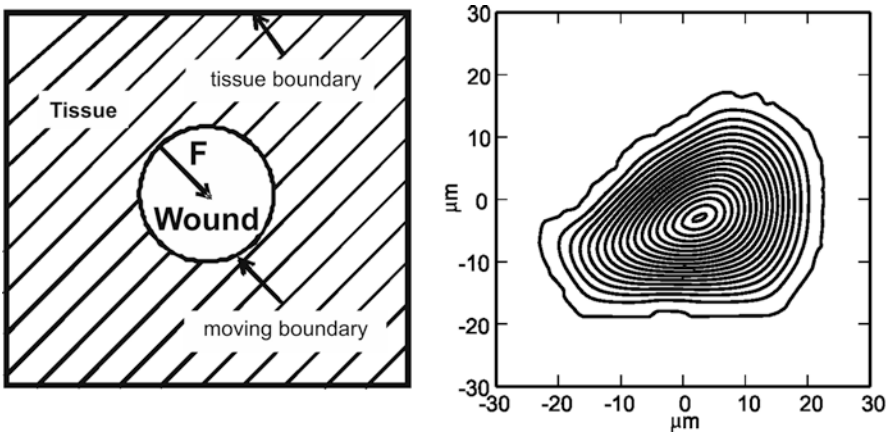

Fig. 11.3 *Left*: schematic of a circular wound surrounded by tissue. The force of the lamellipodia at the edge of the wound is denoted by F. *Right*: model-calculated contours of wound edge (initial position is outermost contour) every 30 min until wound closure

concentration on wound closure during experimental necrotizing enterocolitis. The model predicts low migration speed at high and low integrin concentrations and high velocity at medium concentrations, in agreement with experimental observations [54]. It also predicts that the edge velocity decreases with time, in accord with our experimental observations but contrary to the behavior of reaction–diffusion models. However, the model is only appropriate in situations in which the wound has a simple geometry with two long parallel wound edges. In a follow-up study, Arciero et al. [59] designed a two-dimensional model of cell layer migration that captures the same primary interactions driving the motion of the cell sheet, namely, the elastic coupling between cells in the layer, the adhesion of cells to the substrate, the force generated by lamellipodia both in the interior and at the wound edge, and the proliferation of cells within the layer, but has the additional benefit of being applicable to an arbitrary wound geometry. Figure 11.3 shows a model schematic of a wounded region and the model predicted contours for the closure of an experimental scratch wound at 30-min intervals until the wound is completely closed.

In Arciero et al. [59], the cell sheet is represented as a compressible inviscid fluid, and therefore individual cells are not distinguishable. The leader and follower cells are accounted for in an average manner by including a focused traction force applied by the lamellipodia at the edge of the sheet. The two-dimensional character of the problem requires the use of Eulerian-independent variables. The physical laws governing the mechanics of the layer then yield a partial differential equation problem with moving boundary that is known as the Stefan problem in other contexts [60, 61]. The problem is solved numerically using a level set method, and the basic properties of solutions are analyzed. The model is calibrated for two scenarios: the closure of a wound and the expansion of a cell colony. Parameter values in the model are fit to data from a scratch-wound assay as well as to data from a cell

colony expanding radially outward [9]. Cell proliferation is neglected in wound closure simulations but is included in colony expansion simulations. The model successfully reproduces cell density and edge migration velocity data from both types of experiments.

Cell Signaling Models Models that are developed to understand both the mechanical and biochemical aspects of cell migration can help to determine which phenomena are primarily responsible for initiating cell motility following an injury and what factors regulate the speed and direction of cell migration. In general, the regulation of wound healing by biochemical signals and feedback pathways remains poorly understood. Posta and Chou [62] developed a mathematical representation of ligand-mediated intercellular signaling mechanisms related to the cell migration of epithelial monolayers. Experiments have indicated the need for mitogen-activated protein kinase (MAPK) activation for coordinated cell movement following an injury. The model reproduces two waves of MAPK activity that have been observed experimentally and that may depend on reactive oxygen species (ROS) and competition between a ligand (such as a growth factor) and ROS for the activation of the epithelial growth factor (EGF) receptor. The resulting traveling wave solutions of the model are consistent with MAPK patterns observed experimentally.

Models of the fourth stage of wound healing, i.e., the remodeling of the scar tissue, are primarily concerned with the factors that determine the final size of the scar. Two key features of the scar tissue attract attention: details of collagen composition (relative proportion of type I and type III collagen) and orientation of the fibers. The balance between the two types is regulated by different isoforms of transforming growth factor (TGF)-β protein and was studied by Dale et al. [63] who developed a reaction–diffusion model. The model predicted that different ratios for fetus and adult tissues depend on the secretion of the different isoforms of TGF-β. In a follow-up paper [64], Dale et al. used the model to determine whether fibroblast cells enter the wound area from the surrounding unwounded dermis or from the underlying subcutaneous tissue and gave reasons favoring the latter. The orientation of fibers in the wound tissue was analyzed in a series of papers by Dallon et al. [65] who employ agent-based, as opposed to equation-based, models. In particular, fibroblasts were modeled as discrete entities and the ECM was assumed to be a continuous entity composed of collagen and a fibrin-based blood clot. The following interactions were captured by the model: fibroblasts orient the collagen matrix, fibroblasts produce and degrade collagen, and fibrin and the matrix direct the fibroblasts and determine the speed of the cells. The model was used to predict how multiple cellular phenomena play a role in collagen alignment during wound repair.

Wound contraction is also an important component of wound closure, especially in animals. Contraction is primarily caused by myofibroblasts that exert traction forces on their environment. Experimentally this process has been studied on collagen gels. Contraction was first studied mathematically by Murray et al. [66] who adapted a general model of tissue biomechanics to a wound healing situation. Subsequently, Tranquillo and Murray [67] investigated the interplay between cellular, biochemical, and biomechanical phenomena, which result in wound contraction.

They modeled fibroblast migration and proliferation as well as the deformation of the ECM and formulated an extended model that accounts for the influence of an inflammation-derived mediator on traction, growth, and chemotactic properties of fibroblasts in order to predict the qualitative features of a contracting wound. A similar model was also used by Olsen et al. [68] to study failures in wound closure due to fibroproliferative disorders such as keloid and hypertrophic scars. All of these models describe tissue as a linear viscoelastic material. For embryonic epidermal wound healing, Sherratt [69] developed a model involving actin filament network formation and wound contraction, based on a mechanochemical model for the deformation of epithelial sheets proposed by Murray and Oster [70].

Angiogenesis Models Angiogenesis in a growing tissue has been studied in the context of wound healing or tumor growth. The process of capillary ingrowth is essential to healing since it helps to maintain high levels of metabolic activity by increasing blood supply. The biology of angiogenesis has been studied mostly in the context of cancer growth, but the biology applies equally well to wound healing. Tumor angiogenesis has been modeled by Chaplain and Sleeman [71] and continued by Anderson and Chaplain [72]. Chaplain and Byrne [73] reviewed the similarities of wound healing and tumor growth and Olsen et al. [74] studied the interactions between endothelial cells and soluble regulators (such as growth factors), as well as the insoluble ECM substrate, which consists primarily of collagen. Pettet et al. [75] developed a model of angiogenesis during wound healing that includes contributions of capillary tips, capillary sprouts, fibroblasts, macrophage-derived chemical attractants, oxygen, and ECM. The model reflects the dependence of macrophage activity on local oxygen concentration, which is the major difference between the process in wounds and tumors, and is able to reproduce the failure of wounds to heal when the proliferation rate of endothelial cells is too low. A new version of the model was compared with experimental data by Byrne et al. [76].

Chemotaxis Models The directed movement of cells and organisms in response to chemical gradients, known as chemotaxis, plays an important role in several aspects of physiology, including embryonic development, inflammatory cell migration, wound healing, new vessel formation, and tumor growth. The deterministic Keller–Segel continuum model is a well-established method for representing chemotactic behavior of cell populations since it is able to capture key phenomena that are often lost on discrete or single-cell level models. Hillen et al. [77] analyze ten models that are variations of the Keller–Segel model in order to determine which model components relate most directly to biological observations of chemotaxis. Their analyses include the determination of the existence of model solutions and the identification of long-time behavior of solutions and the form of steady state patterns.

As an example of a chemotaxis model in the context of wound healing, Schugart et al. [78] presented a PDE model of wound healing that focuses on the release of angiogenic growth factors (e.g., VEGF) by inflammatory cells. In particular, the growth factors are assumed to interact with fibroblasts to produce collagen and other components of the ECM, which in turn facilitates the migration of cells into the wound. A circular wound is considered in this theoretical study, and thus the

model is solved over a radial cross section of the wound. Model results suggest that a hypoxic wound environment cannot sustain vascular growth, that hyperoxia promotes wound angiogenesis and healing, and that there is an optimal level of hyperoxia beyond which the beneficial effects of oxygen may be reversed.

Agent-Based Models of Cell Migration

Various types of agent-based models (ABM) have been used to test wound healing hypotheses and to isolate factors that may direct cell sheet migration. Since a detailed description of wound healing ABM is presented in a different chapter of this book, here we focus on ABMs of collective cell migration, as such models are often used as a basis for the development of equation-based models of wound healing.

A detailed model of the dependence of cell speed on adhesion-receptor/ligand binding was proposed by DiMilla et al. [79]. Walker et al. [80, 81] used an agent-based model to simulate the wounded epithelial cell monolayers and suggested that simple rules are sufficient to qualitatively predict the calcium-dependent pattern of wound closure observed in vitro. Khain et al. [82] built upon the work in [80, 81] and considered a simple discrete model, which focused on the effects of three key processes, cell–cell adhesion, diffusion, and proliferation, on wound healing in the context of a scratch-wound assay. Different cell behavior was predicted by the model depending on the adhesion strength and the proliferation rate. In general, the model is defined by a list of rules that dictate the conditions under which cells can proliferate or migrate, depending on the number of nearest neighbors to the cell.

Bindschadler and McGrath [83] used an ABM to simulate cell migration in which cells responded to crowded conditions by decreasing their cell division rates and moving to less crowded areas. The model predictions were consistent with experimental rates of closure. Ouaknin and Bar-Yoseph [84] used the Glazier–Graner–Hogeweg (GGH) model to simulate the collective movement of cells, taking into account adhesion energy, deformation energy, and stochastic behavior of the system. The model results were similar to experimental behavior obtained by Poujade et al. [85], in which leader cells progressed faster than the rest of the cell layer and a fingering morphology emerged. Fozard et al. [86] developed an ABM for epithelial monolayers and used it to derive an equation-based continuum model in the limit of a large number of cells. Relating agent-based and continuum models may help to estimate model parameters and justify model assumptions. Fozard et al. [86] assumed that the energy dissipation of individual cells was due to the drag between the cell and substrate, as well as due to the internal viscosity of the cells (which was not accounted for in the model presented here). Active cell migration and cell division were not included in their model, and a more complex formulation of cell–cell and cell–substrate adhesion could provide additional mechanical insight. The continuum model yielded results consistent with the ABM for even a moderate number of cells. Byrne and Drasdo [87] also derived a continuum model from their ABM for

the growth of cell aggregates on compact monolayers. Growth was assumed to be governed by contact inhibition, and cells were assumed to proliferate. The continuum model agreed with the ABM in the prediction of initial and asymptotic growth regimes for the radius of the colony and the cell population size. A detailed description of agent-based models of wound healing is provided in the next chapter.

Applications of Wound Healing Models

Both equation-based and agent-based theoretical models of wound healing have important applications that extend beyond the context of wound healing. The mechanisms and techniques used to describe migration and proliferation of a cell layer can be used to predict wound closure time as well as to describe the mechanical processes governing morphogenesis, tumor growth, and colony expansion.

Predicting Wound Healing Time Three commonly used methods for estimating wound closure time in clinical practice are the Absolute Area Reduction method, Percent Area Reduction method, and Linear Parameter method [13]. The Absolute Area Reduction method estimates the time rate of change in wound area as the ratio of the difference between the current wound area and original wound area to the total change in time. The Percent Area Reduction method estimates the rate of change in wound area as the difference in wound areas between two consecutive time points. The Linear Parameter method assumes that the average velocity of the wound edge over the wound contour is constant in time and uses the value of a linear healing parameter, which is defined as the ratio of the difference in wound areas to the average perimeter for two consecutive time points, to predict overall closure time for wounds. Recently, Arciero et al. [92] introduced two additional methods for calculating healing time, in which the time rate of change of wound area is not constant but is proportional to the square root (Square Root Method) or the first power (Proportional Area Method) of area. These methods were shown to provide better estimates of closure time than the three previously established methods since they both converge to the correct closure time as more data is available and they provide relatively accurate predictions at early stages of the closure process. While these two methods were shown to be useful for predicting a range of wound healing times for superficial epithelial wounds, other clinical aspects may be required to obtain accurate closure time predictions for wounds of various types and sizes. A comparison of the predicted healing times of these five methods is provided in Fig. 11.4.

Morphogenesis Cell and tissue mechanics are important components dictating embryonic development and organ shape within a body. In particular, at the tissue level, force production and viscoelastic material properties of tissues determine the direction and speed of tissue movements as structures are sculpted. Integrating intracellular force generation with the local micromechanical environment directs

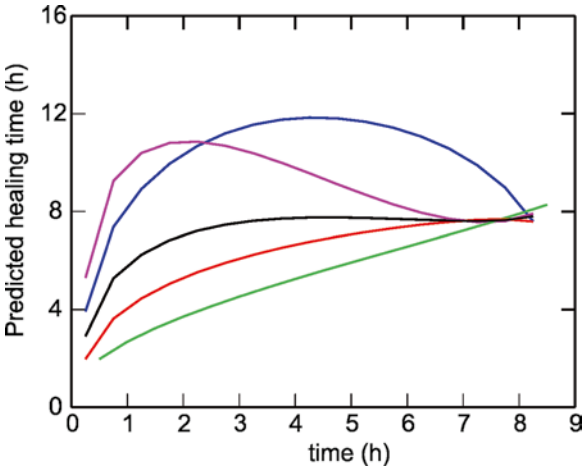

Fig. 11.4 Comparison of predicted healing times for a scratch-wound assay using five different methods: Absolute area reduction method (*green*), percent area reduction (*red*), linear parameter method (*blue*), square root method (*black*), and proportional area method (*magenta*). The predicted healing time is shown as a function of time

molecular–mechanical processes and cell differentiation [88]. Significant advances have been made in morphogenesis experiments, and the use of mechanical and theoretical analyses in this field is beginning to gain momentum. The combination of these experimental and theoretical techniques may help to answer three important questions in the field of morphogenesis outlined by Davidson et al. [89]: (1) are mechanical properties of the embryo important to morphogenesis? (2) at what scale are mechanical properties shaped? and (3) can the processes that generate force be separated from the processes that make tissues stiff?

Tracheal branching morphogenesis and mammary gland development are two examples in which morphogenesis of branched tubular organs or terminal end buds can be studied. Tracheogenesis occurs without mitosis, and thus collective cell migration can be studied in this context without interference from cell proliferation. It has been concluded that the pattern of tracheal branching emerges from the interplay between an extracellular chemoattractant and collective decision making that uses a negative-feedback loop to restrict the number of cells that respond to this chemoattractant [6]. Mammary gland development occurs via the branching morphogenesis of terminal end buds; this branching is unique from most other systems due to the absence of leader cells at the tip of the bud. Instead, the cells at the bud tip for a blunt-shaped multilayered bulb with cells continually exchanging positions [6].

Cancer Several models originally developed for wound healing have been employed to simulate expansive growth and cell migration of tumors [73, 87, 90]. Both discrete and continuous approaches have been used that consider the effects of mitotic inhibitors, nutrient depletion, cell cycle, and new capillary formation on tumor growth [90]. For example, Tracqui [90] developed models that relate cell motility and traction forces and that are used to simulate the transition from a homogeneous distribution of cells on a tumor surface to a nonhomogeneous density pattern that may correspond to a preinvasive stage of the tumor.

Colony Expansion Models for wound healing can be also transformed to simulate the process of cell colony expansion [59]. Trepat et al. [9] recorded the cell density of a canine kidney cell population as a function of distance from the leading edge of the cell layer at 24-h time intervals. Growth of the layer plays a prominent role in the context of colony expansion, and Poujade et al. [85] observed that cell proliferation by a colony of cells occurred almost exclusively within the band where cells were originally seeded, potentially due to the longer presence of cells in the originally seeded region or modifications made by cells to the underlying substrate. When applied to a cell colony scenario, the model in [59] predicts an increase in cell density when approaching the center of the cell colony. The results also suggest that in the experiments of Trepat et al. [9], as in those of Poujade et al. [85], the cells proliferate only in the region originally seeded by the cells.

Conclusions

A multitude of mathematical models of wound healing have been developed in the attempt to understand the qualitative and quantitative aspects of the process. Although many of the models differ substantially in scope, the mechanical and mathematical principles underlying all of the models are related and can be applied to multiple biological systems. The choice of model type depends on the information desired. Certain models are appropriate at a cellular level (e.g., to simulate individual cell motion), while other models are more beneficial on a tissue level (e.g., to represent collective migration).

The study by Stolarska et al. [91] provides a perfect example of differentiating among model types while also highlighting model similarities. In the study, three different cell and tissue mechanics models are presented: a continuous model of an arbitrarily deformable single cell, a discrete model of the onset of tumor growth, and a hybrid continuum–discrete model of later stages of tumor growth. Three essential processes involved with cell migration are captured in the single cell model: the controlled spatiotemporal remodeling of the actin network, the generation of traction forces to move the cell body, and the construction and destruction of focal complexes or focal adhesions. Cell-level details are incorporated into their tissue-level model, including how an individual cell reacts to forces on it, how cells interact mechanically with their surroundings, how growth and division are described, and how stress affects growth. And thus, predictions obtained across multiple levels of mathematical modeling can be used to gain insight into wound healing processes.

Byrne and Drasdo [87] compare the benefits of using a biophysical agent-based or a continuum mechanical model to track the expansion and migration of cells in a dense monolayer. Single-cell-based models permit a higher degree of spatial resolution than models composed of locally averaged quantities; however, large cell population sizes are not amenable to investigation using agent-based models. Ultimately, conditions under which spatiotemporal behavior of the different models agreed were identified in order to determine how to relate the parameters in the different

models. The same growth pattern for dense and sparse cell aggregates was obtained using both models.

Khain et al. [82] commented that most theoretical models of wound healing employ reaction–diffusion equations for the cell density and a growth factor. However, in their study, they demonstrated that simple discrete models can be applied to wound healing and yield the results obtained from reaction–diffusion equations when proliferation is small. Since biologically reasonable rates of perfusion are small compared to rates of diffusion, both continuum and discrete models provide good predictions of the velocity of a wound edge.

Whether an ODE, PDE, or ABM wound healing model is used to describe the migration of cells in response to an injury, all three model types aim to accomplish three main objectives: to track the cell response and position following the induction of a wound, to understand the role of tissue growth factors in the healing process, and to predict the time required for a wound to heal. As described in this chapter, the particular choice of theoretical wound healing model dictates the specific phenomena or elements that are most likely to be understood and uncovered.

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