Mathematical Modeling Can Advance Wound Healing Research

Shakti N. Menon,[1,*] and Jennifer A. Flegg[2]

[1]The Institute of Mathematical Sciences, Chennai, India.
[2]School of Mathematics and Statistics, University of Melbourne, Melbourne, Australia.

Shakti N. Menon, PhD

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*Correspondence: The Institute of Mathematical Sciences, CIT Campus, Taramani, Chennai 600 113, India
(e-mail: shakti@imsc.res.in).

Significance: For over 30 years, there has been sustained interest in the development of mathematical models for investigating the complex mechanisms underlying each stage of the wound healing process. Despite the immense associated challenges, such models have helped usher in a paradigm shift in wound healing research.
Recent Advances: In this article, we review contributions in the field that span epidermal, dermal, and corneal wound healing, and treatments of nonhealing wounds. The recent influence of mathematical models on biological experiments is detailed, with a focus on wound healing assays and fibroblast-populated collagen lattices.
Critical Issues: We provide an overview of the field of mathematical modeling of wound healing, highlighting key advances made in recent decades, and discuss how such models have contributed to the development of improved treatment strategies and/or an enhanced understanding of the tightly regulated steps that comprise the healing process.
Future Directions: We detail some of the open problems in the field that could be addressed through a combination of theoretical and/or experimental approaches. To move the field forward, we need to have a common language between scientists to facilitate cross-collaboration, which we hope this review can support by highlighting progress to date.

Keywords: mathematical modeling, wound healing, scratch assays, fibroblast-populated collagen lattices

SCOPE AND SIGNIFICANCE

With this review, we intend to illustrate how wound healing models can be developed, given a set of assumptions, how they can be used to make predictions that either agree with existing data, or which can be tested with new experiments, and how newer models can build upon existing models by incorporating more details of biocomplexity. We explain how advances in this field are contingent on a common language between mathematicians/statisticians and biologists/clinicians, and highlight the benefits of further interdisciplinary collaboration.

TRANSLATIONAL RELEVANCE

The mathematical modeling cycle is illustrated in Fig. 1, whereby a real-world problem is simplified to a working model before being represented in equation form. Such models provide a testbed for exploring the roles played by the individual components underlying the healing process in question, and have the potential to generate theoretical predictions that could not

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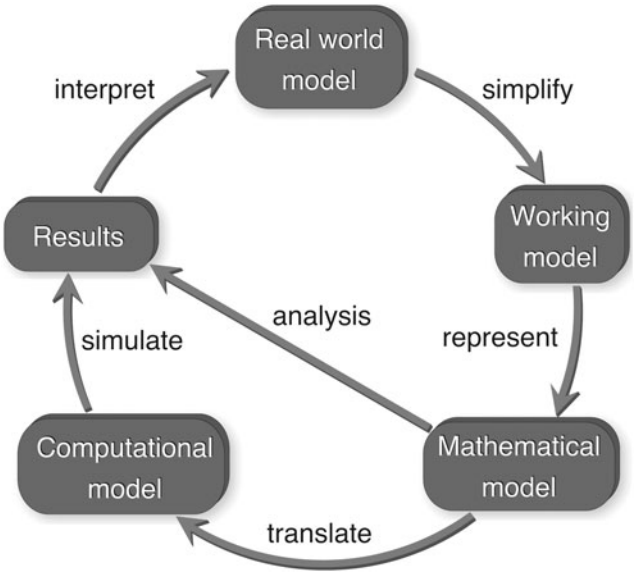

Figure 1. Flow chart presenting a schematic overview of a typical modeling cycle. Starting with a real-world model of a physical/biological process, the first challenge is to identify the key mechanisms that underlie the observed phenomena and obtain a simplified working model. This can subsequently be represented as a mathematical model by expressing the interdependencies of the constituent components in terms of the appropriate theoretical framework (*e.g.,* a model of coupled differential equations), accounting in each case for the spatiotemporal changes that would arise through diffusion, proliferation, and other processes. Depending on the complexity of the resulting model, it can either be analyzed directly, or be translated into a computational model (through appropriate discretization schemes) that can be numerically simulated. In either case, an interpretation of the obtained results can be used to refine the real-world model by providing insights into the physical process under consideration, and be used to make predictions that can be compared with empirical observations. Note that the modeling cycle will be situation dependent, as each phase may constitute several intermediate steps, depending on the process under consideration and the modeling approach being adopted.

have been anticipated otherwise, thereby stimulating further biomedical research and reducing the need for difficult and costly experiments. In addition, they provide a means to identify elements of the healing process that can be manipulated in a rational, mechanism-based strategy.

CLINICAL RELEVANCE

Wound healing remains one of the biggest challenges for public health systems worldwide, with total spending for all wound types estimated to be up to $96.8 billion per annum in the United States alone.[1] Nonhealing wounds are a source of considerable pain, immobility, and a decreased quality of life for those who suffer from them.[2] While *in vivo* studies are the gold standard for assessing the effect of novel treatments of wounds, mathematical modeling can play an important complementary role in addressing certain questions that lie beyond the scope of current experimental techniques.

DISCUSSION OF THE MATHEMATICAL MODELING OF WOUND HEALING

In this section, we discuss some of the most influential mathematical models that were developed to describe wound healing. While some of these studies intended to capture aspects of the underlying biology, others aimed to make novel predictions by describing phenomena that may be difficult to investigate experimentally. Moreover, as we shall discuss later, models have also been used to probe the efficacy of different therapies for wound healing, or to complement *in vitro* experiments. To elucidate why certain models could yield more accurate predictions than previous approaches, we highlight the underlying assumptions in each case.

Epidermal wound healing

The process of epidermal wound healing is relatively simple and primarily involves reepithelialization, in contrast to dermal wounds that heal through contraction and remodeling. This has facilitated the development of theoretical frameworks that can capture observations from associated experimental studies. One of the first mathematical models of epidermal wound closure was developed by Sherratt and Murray.[3] They conjectured that if the wound was shallow, then it would suffice to describe the activity over a two-dimensional (2D) spatial domain, and furthermore, if the wounded region was initially circular, then one could simply focus on the changes along the radial direction. Their resulting model consisted of a pair of coupled partial differential equations that described the spatiotemporal changes in the densities of epidermal cells and a generic diffusible chemical that regulates cell proliferation. Typical numerical solutions of this model are shown in Fig. 2, illustrating the differences that arise when cellular random motion is modeled using different assumptions regarding the nature of diffusion. Simulations of their model were consistent with earlier experimental data for the change in the relative radius of epidermal wounds.[4] Despite the minimal assumptions underlying this model, the approach that was pioneered in this study was highly influential on numerous subsequent models of wound healing, as discussed later.

A salient feature of the mathematical modeling approach is that it can often point toward new avenues of exploration. For example, upon developing a set of models[5] for the role of keratinocyte growth factor (KGF) in epidermal wound healing, Wearing and Sherratt suggested that KGF may play a broader role than enhancing the speed of reepithelialization.

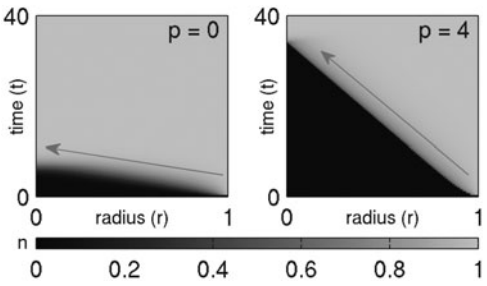

Figure 2. Results obtained from numerical simulations of the model by Sherratt and Murray.[3] This model described the process of reepithelialization in a circular epidermal wound of radius r where the parameter p mediated the migration of cells over time (t). Specifically, cells were assumed to migrate through diffusion that occurs in a linear ($p=0$) or nonlinear $p>1$ manner. It was assumed that the normalized density of epidermal cells (n), whose value is indicated by the color bar, was initially zero inside the wound, and was at a maximum at the wound edge ($r=1$). The two *panels* display the change in the cell density as a function of r for two different values of p, illustrating the changing rate of migration from the wound edge to the interior. The *arrows* in each subplot indicate the movement of the wave front of cells.

KGF, which is part of the family of fibroblast growth factors,[6] upregulates the proliferation of keratinocytes, and is produced by fibroblasts in the dermis.[7] It has been observed that KGF expression levels are lower during pathological wound healing,[8] while the application of KGF was found to enhance the rate of wound closure.[9] Wearing and Sherratt described the paracrine signaling mechanism of KGF by considering the dermal and epidermal concentrations of this growth factor, as well as the number of free and bound receptors per basal epidermal cell.[5] Their modeling revealed that the large increase in KGF levels observed immediately after wounding was above the range required for an optimal rate of wound closure, leading to their conclusion that KGF plays additional important roles during this process. In addition to the aforementioned studies, there have been several other attempts to mathematically model aspects of wound closure and, as we discuss later, many of these have gone hand in hand with *in vitro* experiments.

Corneal epithelial wound healing

In comparison to skin wound healing, the process of corneal epithelial wound healing is relatively simple.[10] It comprises four distinct phases[11]: an initial *latent* phase that lasts a few hours, in which epithelial cells adjacent to the wound undergo a phenotypic change to a more motile form, a subsequent *migration* phase that involves the movement of these cells into the wound, followed by a *proliferation* phase in which the epithelium is replenished through increased mitotic activity at the wound margin, and a final *attachment* phase in which the new cells adhere to the basement membrane. Figure 3 illustrates the lateral migration and proliferation of epithelial cells that occur during corneal wound healing.

A key component in this process is epidermal growth factor (EGF)—a large protein that is the main regulator of epithelial repair[12,13] and, when applied topically, is known to stimulate reepithelialization.[14] The role of EGF in promoting corneal wound healing was first investigated by Dale *et al.* using a model for the interaction between a population of a single cell type and a chemical species (representing EGF) along one spatial dimension.[15,16] Using parameter values that were estimated from experiments, and assuming a constant rate of production of EGF, the speed of reepithelialization predicted by their model closely matched experimental observations for the healing rates of corneal wounds. Their model simulations suggested that while EGF does not strongly regulate cell mobility, it plays an essential role in enhancing and regulating mitosis. Moreover, while EGF is known to be present in the tear film overlying the epithelium,[17] their results indicated that, for corneal wound healing to proceed at the expected rate, an additional source of EGF was required. They suggested that EGF may also be released from the exposed wounded tissue, and could facilitate inward migration of cells into the wound.

Although the topical application of EGF has been shown to enhance corneal epithelial wound healing in animals,[18] it was observed to be much

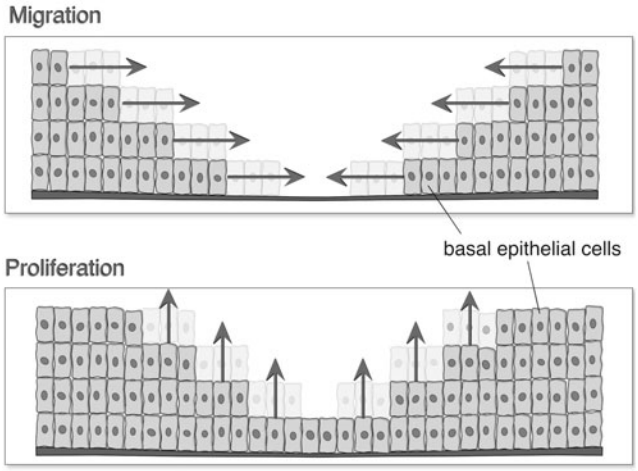

Figure 3. Schematic illustrating the two main processes that occur during corneal epithelial wound healing. Basal epithelial cells from the edge of the wound migrate inwards to seal the gap in the damaged area. Subsequently, cells in the interior of the wound proliferate through mitosis to replenish the wounded region.

less efficacious in human patients.[19] Sheardown and Cheng hypothesized that this may be due to nonoptimal EGF delivery profiles, and developed a mathematical model to simulate how the profile might affect the epithelial healing process.[20] Their model was an adaptation of the model for epidermal wound healing by Sherratt and Murray[3] and described the time evolution of cell density and EGF concentration along a single spatial dimension. They experimentally investigated the migration of cells in cultured rabbit corneal epithelial cells, and their observations closely agreed with the results obtained using their mathematical model. Furthermore, their results yielded predictions for three treatment scenarios: one where EGF is not added to the wound, one where EGF is delivered from topical eye drops, and one where EGF continually perfuses into the wound. Their results suggested that the optimal delivery profile would involve a continuous exposure to EGF. They proposed that such a theoretical framework could help minimize the necessity of *in vivo* wound healing experiments in animals, and could help in designing improved controlled release systems.

While the model by Dale *et al.*[15,16] could predict wound healing speeds in corneal epithelial wounds, it was not suited to predict cell kinetic data as, in contrast to experimental observations, it predicted that the mitosis rate increases as one approaches the wound center.[21] It was proposed that this discrepancy could be accounted for using a modified model that considered two cell types[21]: proliferative and quiescent, with only the former capable of undergoing mitosis. This model incorporated different migration rates for the two cell types and it was assumed that cell division was upregulated by juxtacrine signaling, as such a mechanism is known to play an important role in epidermal wound healing.[22] Simulations of the model captured experimental data for the mitotic rates in the corneal epithelium of rats (specifically Refs.[23,24]), at various spatial positions and at different times after wounding.

Another intriguing aspect of corneal epithelial wound healing is that cell migration at the wound edge has been observed to be influenced by small electric fields that arise due to the fact that the transcorneal potential difference is zero inside the wound, but is nonzero at the wound edge.[25,26] This was modeled by including an additional transport term in the equation for cell density, such that cells exhibit electrotaxis in the presence of an electric field,[27] and simulations revealed that the speed of wound healing depends linearly on the strength of the electric field.

Dermal wound healing: the proliferation stage

Wound healing angiogenesis. A crucial phase of the proliferation stage of healing is angiogenesis, which involves the development of new blood vessels from preexisting ones. During this time, endothelial cells (ECs, which line the walls of vessels) undergo rapid proliferation (for an extensive review of the mathematical models of wound healing angiogenesis, see Refs. 28, 29). Pettet *et al.* developed two of the first models of wound healing angiogenesis, which described the interactions between three[30] and six[31] species, respectively. In these models, the chemotaxis of capillary tips and the laying down of new blood vessels were described using the "snail-trail" mechanism that was initially proposed in the context of tumor-induced angiogenesis.[32] Consistent with experimental observations, they observed that low oxygen regions are needed to stimulate healing and that excess oxygen will prevent further angiogenesis. Results obtained from typical numerical simulations are illustrated in Fig. 4, displaying the development of new capillaries, which are attracted toward regions of low oxygen (and high chemoattractant) concen-

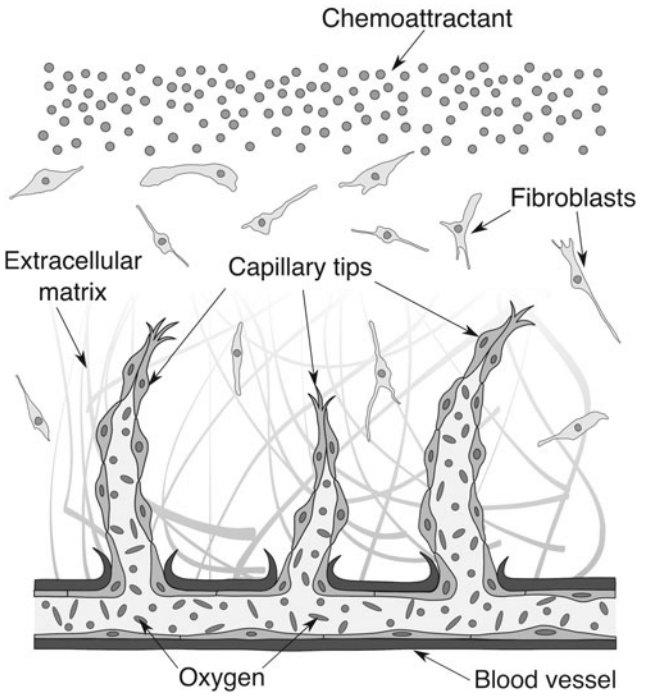

Figure 4. Schematic illustration of the key components of the model of wound healing angiogenesis developed by Pettet *et al.*[31] This model explicitly described the migration of capillary tips through the extracellular matrix by nonlinear diffusion and chemotaxis toward a chemoattractant (namely macrophage-derived growth factors). They modeled the production of collagen by fibroblasts, which also exhibited chemotaxis. In addition, their model accounted for the role of oxygen concentration on the production of chemoattractant by macrophages.

tration. Contemporaneously, Olsen modeled this by taking into account haptotaxis and haptokinesis (random migration mediated by the extracellular matrix [ECM]) of ECs.[33] Their model predicted that ECM-mediated random motility and cell proliferation are key processes that drive angiogenesis. The three-species angiogenesis model[30] was later extended to a circular wound geometry by Byrne *et al.*, who calibrated the model to experimental data on the area of normal and chronic wounds over time.[34] Their results suggested that the response of capillaries to angiogenic factors, the production rate of angiogenic factors, and/or the proliferation rate of the cells at the capillary tips may be responsible for failed healing of wounds. Later, it was demonstrated that key qualitative features of wound healing angiogenesis, such as the propagation of a structural unit into the wound center, could be captured in a model containing just two species (ECs and blood vessels).[35]

Other approaches include a description of the movement of ECs through random walks[36] that used a modeling framework proposed in the context of tumor-induced angiogenesis.[37] Contemporaneously, Vermolen and Javierre developed a mathematical model for contraction, angiogenesis, and closure during the healing of cutaneous wounds.[38] Their model was able to confirm the clinical observation that wound closure proceeds by keratinocytes crawling and climbing over each other during the early stages of healing, whereas in the later stages, the cells form layers parallel to the skin surface. The same problem has also been approached by modeling the skin as a hyperelastic material,[39] where simulations revealed that an elliptical wound vascularizes 2 days earlier than a circular wound, but both experience a similar level of contraction level during that time (a 25% reduction in size).

Collagen–fibroblast interactions and the role of growth factors. Subsequent to the neovascularization of the wound area, the provisional fibrin mesh synthesized by the recruited fibroblasts is replaced with a transitional collagen matrix known as granulation tissue,[40] a process that is enhanced by the presence of transforming growth factor-β1 (TGF-β1).[41] During this stage, fibroblasts secrete type-III collagen, which is organized into loose bundles.[42] In turn, this collagen matrix acts as a scaffold that fibroblasts use to move across the wound.[40] Consequently, the interactions between fibroblasts and collagen fibers play a significant role in mediating the quality of the resulting scar.

One of the first mathematical models to explicitly incorporate these interactions was developed by Olsen *et al.*,[43] who took into account the observations that the movement of cells through the ECM caused a change in the orientation of fibers,[44] and that cells move preferentially along the direction in which the underlying fibers are oriented.[45] In their model, cells synthesize, degrade, and reorient ECM, which in turn mediates the proliferation, haptotaxis, and haptokinesis of the cells. While this anisotropic model of wound healing was the first to take into account the effect of fibroblasts on collagen alignment (and *vice versa*), a contemporaneous model by Dallon and Sherratt[46] explicitly allowed fibroblasts and collagen to be oriented at any angle in a 2D plane (in contrast to Ref.43, in which fibers are aligned along one of two perpendicular directions). Their model accounted for the random reorientation of fibroblasts, as well as their preferential reorientation along the direction of collagen fibers, and *vice versa*. However, this model did not consider the change in collagen density or the motion of fibroblasts, and did not account for the spatial heterogeneity in orientation. Nevertheless, these models provided a useful theoretical framework for several subsequent models of collagen–fibroblast interactions during wound healing.

For instance, Dallon *et al.* built upon these studies to develop a model that described collagen fiber orientation, and fibroblast cells as discrete units that move across the wound.[47] The motion of the fibroblasts was controlled by a single parameter that mediated contact guidance, that is, the extent to which the direction of cell movement was influenced by the orientation of underlying fibers (Fig. 5). They also extended this model to include two different types of fibers, namely collagen and fibrin, where the latter constitutes a blood clot that the fibroblasts degrade and replace with a collagen network. Their model predicted that alignment could be enhanced by increasing the speed of cells, and that a reduction in contact guidance could lead to a lesser extent of alignment. In addition, they observed that the rate of production of ECM by fibroblasts does not significantly affect the alignment of the resulting fibers. As we shall discuss later, this modeling framework has been used to investigate the conditions underlying excessive scarring.

They subsequently extended this model to make the additional assumption that the cell speed was dependent on the (time varying) concentration of TGF-β, which was assumed to be spatially homogeneous.[48] The change in collagen matrix alignment was simulated over the course of the first 240 h after wounding, using earlier data for the change in TGF-β concentration.[49] Their results

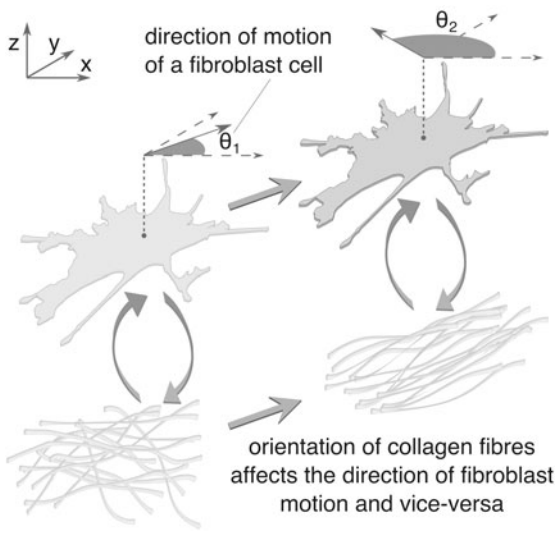

Figure 5. Schematic illustration of the model of wound tissue reorganization by Dallon *et al.*[47] The model describes the interactions between discrete fibroblast cells, which migrate across the wound, and the underlying collagen matrix, described through a vector field. At a given moment in time, the direction of motion of a fibroblast (say, θ_1) is modulated (to a new angle θ_2) by the orientation of proximal collagen fibers, which in turn are reoriented by the fibroblast.

suggested that, while TGF-β may almost double the cell proliferation rate, this does not impact the alignment of the resulting fibers. Furthermore, they found that collagen alignment patterns were only slightly influenced by the changes in cell movement and collagen production in the presence of TGF-β. However, TGF-β (in particular, the TGF-β isoform) is also known to regulate the filopodia extensions of cells,[50] and when they modeled this by allowing the cells to change their direction more frequently, they observed that the resulting fiber alignment was in agreement with experimental observations.

McDougall *et al.* later extended this work to consider the growth factor-mediated chemotaxis of fibroblasts into the wound area.[51] They modeled this by incorporating a generic chemoattractant that is produced in the wound, comprising a fibrin-based ECM, and which diffuses into an unwounded area comprising a collagen-based ECM and fibroblasts. The speed and direction of individual fibroblasts were assumed to depend on the local gradient of chemoattractant. Furthermore, in accordance with observations that fibroblast density varies across the depth of the dermis, it was assumed that the initial cell density profile varied linearly over space. They observed that the resulting orientation of collagen fibers was strongly related to the chemoattractant gradient. In addition, they predicted that the spread of the chemoattractant profile may influence the extent to which the fibers of the scar and surrounding tissue would be cross-linked.

Apart from TGF-β, several other growth factors play crucial roles during dermal wound healing. For instance, platelet-derived growth factor (PDGF), which is produced in the initial fibrin clot, acts as a chemoattractant for fibroblasts.[52] This chemotactic process was investigated using a model that incorporated a description of the phosphoinositide 3-kinase signaling pathway,[53] in which it was assumed that PDGF degrades spontaneously, and is consumed by fibroblasts through receptor-mediated endocytosis. Simulations of this model revealed that a constant PDGF gradient could be maintained at the leading front of fibroblasts, and that the relative steepness of the gradient did not significantly affect the rate of chemotaxis.

In addition to stimulating chemotaxis and mediating the proliferation of fibroblasts, growth factors play an important role in the crosstalk of fibroblasts and keratinocytes during dermal wound healing. This was investigated by Menon *et al.*, who developed a 2D model to investigate the roles of TGF-β, PDGF, KGF, and interleukin-1 during this crosstalk, and its effect on collagen production under conditions of normal healing, prolonged inflammation, and chronic hypoxia.[54] It was observed that the prolonged presence of TGF-β in the wound led to an excess production of collagen, while collagen production and reepithelialization are greatly reduced under conditions of chronic hypoxia.

Dermal wound healing: the remodeling stage

Upon completion of the proliferation phase, the healing process enters the final remodeling phase that can last for several months or years.[55] This stage is initiated by the replacement of the granulation tissue—a slow process that requires the continual synthesis and degradation of collagen.[40] Specifically, type-III collagen is replaced by type-I collagen, whose fibers occur in dense parallel bundles, in contrast to the basket weave of fibers observed in healthy skin.[42] Concurrently, fibroblasts receive external cues that cause them to differentiate into more contractile phenotypes, protomyofibroblasts, and myofibroblasts,[56–58] which attach to the collagen matrix and facilitate the contraction of the wound.[59] While the granulation tissue is initially highly vascular,[42] angiogenesis gradually ceases and the tissue evolves into an avascular scar that regains at most 80% of the strength of the original tissue.[60]

During this period, the tissues are "remodeled": they undergo mechanical restructuring, while their physical properties (such as anisotropy and stiffness) concurrently evolve.[61] The remodeling process affects the mechanical stresses experienced by cells within the wound, and a dysregulation of this process may lead to pathological scarring.[60,62] Hence, it is crucial to understand the interplay of mechanical stresses and chemical signaling during this phase of wound healing. To this end, we discuss two types of "mechanochemical" approaches to describing the contracting tissue, one in which the ECM is described as a linear viscoelastic material, and the other that uses the framework of "morphoelasticity" to capture changes associated with tissue remodeling in relationship to a hypothetical co-evolving reference state.

Describing the ECM as a viscoelastic material. One of the seminal models of wound contraction was developed by Tranquillo and Murray,[63] who built upon earlier work for modeling cell traction[64] and introduced a "mechanocellular" or "mechanochemical" framework for describing cell-level behaviors (such as proliferation and migration), along with the mechanical behavior of the ECM. Their model included equations for the conservation of mass of fibroblasts and ECM, which, due to the contraction of the tissue, each undergo passive convection with the same velocity. In addition, they described the cell/ECM composite as an isotropic viscoelastic material (having both viscous and elastic properties). Using this model, they obtained close agreement with the experimental observations of McGrath and Simon[65] for the contraction of full-thickness wounds in rats. Specifically, their model predicted that the contraction rate constant, as well as the ratio of final to initial wound area, does not strongly depend on either the geometry of the wound or the initial area.

This modeling framework was highly influential on several subsequent models of wound contraction, including a mechanochemical model of the behavior of fibroblasts, myofibroblasts, and a generic chemical species (mainly representing the activity of PDGF, but also other growth factors such as TGF-β), as well as the ECM, which was assumed to be a linear viscoelastic material.[66] Simulations of this model were in close agreement with the experimental observations of McGrath and Simon,[65] as well as measurements for the clearance of PDGF from the wound center over the course of contraction.[67]

A contemporaneously developed mechanochemical model of wound contraction accounted for the strain and the concentrations of collagen inside and outside the wound.[68] Upon changing a single parameter, this model could be used to describe situations where there is no plasticity, as well as a nonlinear elastoplastic case. Once again, the predictions of this model were consistent with the observations of McGrath and Simon.[65]

Morphoelasticity and its application to wound healing. Any theoretical framework proposed to describe wound contraction needs to take into account the fact that the "reference state" of the system in question (*i.e.*, the state it would revert to upon removal of all internal stresses) will continually change due to the concurrent remodeling of the tissue. This relates to a concept developed in the context of describing the mechanical properties of biological tissues, referred to as the multiplicative decomposition of the deformation gradient,[69,70] which can be understood as follows: as shown in Fig. 6, if F represents the deformation gradient from a tissue's reference state to its current (stressed) state, then it can be expressed as $F = AG$, where the plastic/growth component G represents the deformation from the reference state to a hypothetical "zero-stress state" (in which all internal stresses are relieved) that evolves over time, and A represents the deformation from this zero-stress state to its current state (see Menon *et al.*[71] for a recent discussion of this concept). Note that if a tissue were purely elastic, then the zero-stress state would be

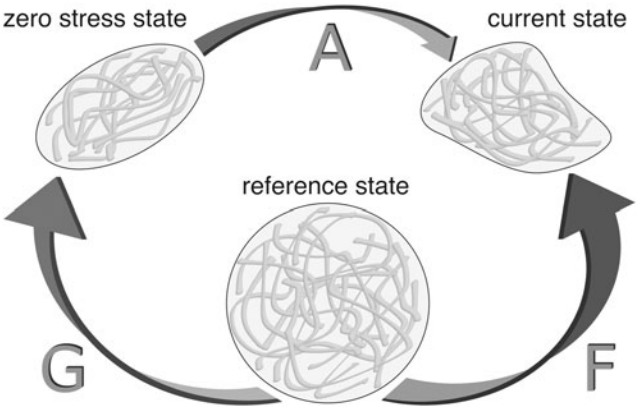

Figure 6. Relationship between the states of a morphoelastic object whose internal structure is continually reorganized. Upon being subjected to an external force, represented by the deformation gradient tensor *F*, the object evolves from its initial state. However, upon the removal of this force, the object does not revert back to its reference state due to the changes in its internal properties. This is represented in terms of the evolution of the zero-stress state of the system, where *G* represents the associated deformation from the reference state. Thus, the current stressed state of the system can alternatively be viewed as a deformation *A* from the zero-stress state.

the same as that of the reference state. This view of the mechanical properties of tissues is significantly different from classical plasticity, and Goriely and Ben Amar proposed the term "morphoelasticity" for describing the combination of changes (elastic and plastic) that occur in such tissues as a consequence of growth and remodeling.[72] A morphoelastic description of a biological tissue would take into account changes arising from tissue growth as well as internal energy, and would incorporate the coevolution of the mechanical state of the tissue as well as the densities of cells, and other relevant properties. The framework of morphoelasticity has been used to model the role of TGF-β in stimulating fibroblasts to differentiate into myofibroblasts in contracting wounds,[73,74] wound contraction and scar formation,[75] as well as dermal wound closure.[76] More recently, as we discuss later, this theory has been applied to describe the fibroblast-induced contraction of collagen lattices observed in *in vitro* experiments.[71]

Pathological wound healing

As wound healing is a tightly regulated process, dysregulation in any of the stages can lead to pathological outcomes that range from excessive scarring to nonhealing wounds. These may arise due to a breakdown in the signaling mechanisms underlying the production of collagen and other components, or from environmental factors such as excessive mechanical stress. Below we discuss some of the treatments of pathological wounds, and the attempts to mathematically model them.

The role of oxygen in mediating the healing process. It is well accepted that oxygen is a crucial component of the wound healing process.[77,78] This has led to research into the use of oxygen-based therapies in wound healing, including hyperbaric oxygen therapy (HBOT) for the treatment of chronic wounds (see reviews[79–81]), supplemental oxygen to reduce the risk of wound infection,[82,83] and topical oxygen as an adjunct therapy for wound healing.[84]

Nonhealing or chronic wounds often fail to heal due to a lack of oxygen in the wound site and hence many mathematical models have focused on how the healing process can be stimulated, particularly through the supply of additional oxygen. Simulations of models developed to investigate the role of tissue oxygen tension on wound healing suggested that supplemental oxygen therapies can stimulate angiogenesis and support healing.[85] Results obtained from a model for ischemic dermal wounds suggested that ischemia may impair wound closure by limiting macrophage recruitment.[86]

One of the first models for assessing the effect of HBOT on the healing of chronic wounds was developed by Flegg *et al.*,[87] who observed that intermittent HBOT may stimulate healing in chronic wounds, while normobaric oxygen would be ineffective in such cases. In later work, asymptotic methods were used on a simplified version of the model to establish conditions under which angiogenesis will be initiated in terms of model parameters, including the rate of oxygen supply and consumption.[88] By extending their earlier work, they were able to show that, under their model, intermittent HBOT can accelerate the healing of a diabetic wound, but that sessions should be continued until complete healing is observed.[89] Simulations revealed that HBOT did not improve healing for normal wounds, and that fewer, longer sessions of oxygen were not an effective treatment option. Subsequently, Flegg *et al.* developed a mathematical model to investigate the healing of venous ulcers under short-stretch and three-layered bandages that support the delivery of oxygen from the surrounding healthy tissue.[90] The geometry of this model and moving wound boundary that was fitted to clinical wound data is illustrated in Fig. 7. With simulations, they were able to predict that the three-layered bandage results in

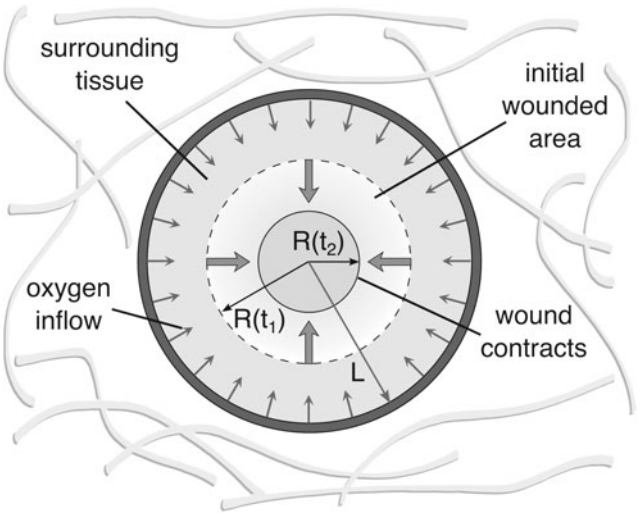

Figure 7. Schematic illustration of the model of healing venous ulcers by Flegg *et al.*[90] The model describes the contraction of a circular wound of radius $R(t)$ whose edge moves toward the center at a rate that depends on the available concentration of oxygen, which enters the wound from blood vessels in the surrounding unwounded tissue. The blood vessels are assumed to be located at a distance L from the wound center. This model was used to investigate how the healing rate varied under two different compression therapies.

faster healing than the short-stretch bandage as it allows more oxygen to flow into the wound, and that the difference between them is more significant for wounds of larger initial area.

Modeling approaches have also had implications for diabetic wound healing. For instance, Waugh and Sherratt developed an ordinary differential equation (ODE) model, and predicted that the distribution of macrophage phenotypes may be altered in diabetic wounds compared to normal wounds.[91] They later extended their model to simulate the effect of engineered skin substitutes on diabetic wounds and found that the therapy works by increasing the amount of hyaluronan in the wound environment.[92]

Such ODE frameworks have also been used in a model of impaired healing in hypoxic wounds that helped identify possible therapeutic targets, including the fibroblast death rate and rate of fibroblast recruitment,[93] and in a model of the impact of local oxygen level on collagen accumulation, which revealed that increased fibroblast proliferation is more effective at bringing about wound closure than antibiotics.[94]

Fibroproliferative disorders and the role of TGF-β. An excessive deposition of collagen during the proliferation stage can lead to the formation of hypertrophic scars and keloids.[60] These fibroproliferative disorders differ in terms of the rate of collagen synthesis, as well as the thickness of the resulting collagen fibers.[42] While there is still ongoing work into the causes of such disorders, it has been suggested that pathological scarring is related to the extent of the inflammation stage of healing.[95] This view is supported by the fact that scar formation is not observed in early fetal wound healing,[96] a process that is characterized by a lack of inflammation.[97] Fetal skin is also characterized by relatively low concentrations of TGF-β1, which is expressed at high levels in adult wounds and which can induce scarring when added to fetal wounds.[98] This has generated interest in the role of growth factors such as TGF-β1 on scarring, and several mathematical models have been proposed to describe the conditions that may lead to pathological scars.

One of the first attempts at modeling the conditions that underlie fibroproliferative disorders was by Olsen *et al.*,[99] who, upon adapting their earlier mechanochemical model of wound healing,[66] obtained results that suggested the rate of production of growth factors by cells plays a significant role in determining whether one may observe excessive wound scarring. However, their model did not explicitly describe the effect of TGF-β, and assumed a generic chemical that represented the combined effect of a number of growth factors. A contemporaneous detailed model for collagen synthesis during dermal wound healing in adult and fetal wounds[100] explicitly accounted for the role of two isoforms of TGF-β (namely, TGF-β1 and TGF-β3) in both their latent and active states, as well as the production of both collagen-I and collagen-III from their respective procollagens, and their degradation by associated collagenases. The densities and ratios of collagen-I to that of collagen-III obtained from simulations of this model agreed with known experimental observations. Furthermore, the addition of TGF-β1 at early stages of the healing process was observed to yield higher levels of collagen-III, while the initial addition of TGF-β3 led to levels of collagen-III that were consistent with that of normal skin. Thus, this model predicted that the early addition of TGF-β3 may help reduce scarring in dermal wounds.

Later, Cumming *et al.*[101] built upon earlier studies[47,51] to develop a 2D hybrid model of dermal wound healing. They considered the network of fibers in the initial fibrin clot, as well as the collagen network that is formed and remodeled by fibroblast cells that migrate into the wound in response to TGF-β produced by macrophages (Fig. 8). They assumed that fibroblasts and macrophages are initially present in the unwounded area surrounding the wound, and explicitly modeled collisions and contact inhibition. Cells were assumed to move by a stochastic chemosensory mechanism, based on the binding of cytokine molecules to (a finite number of) receptors on the cell. Results obtained by this model confirmed observations from previous investigations that a reduction in the production of TGF-β or a decrease in its diffusion rate can significantly impact the production of collagen. Moreover, they observed that a reduction in scarring can be obtained by explicitly blocking the number of available cytokine receptors.

There have also been investigations into the factors underlying fibroproliferative disorders, such as nitric oxide (NO), which suppresses the transition from fibroblasts to myofibroblasts.[102] Cobbold and Sherratt developed a model that described the NO-mediated production of collagen by wound fibroblasts in hypoxic conditions,[103] which they used to describe how the final scarring outcome (normal, hypertrophic, or keloid) depended on the initial collagen density and the production rate of NO. In addition, they developed a more detailed model that accounted for the additional roles of macrophages, TGF-β, and blood vessel density.

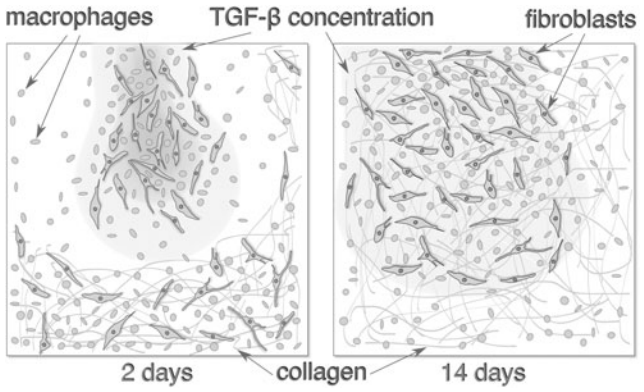

Figure 8. Schematic illustration of results obtained by Cumming *et al.*[101] using their model of scarring. The model describes the interactions between discrete fibroblast cells, which migrate toward a source of chemoattractant (TGF-β), and the underlying matrix of collagen fibers. The model also considered the role of macrophages, which migrate into the wound space upon being chemotactically attracted by TGF-β and which, on contact with fibrin within the wound, synthesize additional TGF-β. The displayed panels recapitulate the key results of fig. 8 of Cumming *et al.*,[101] which shows the evolution of the species of the model at different days after the initial wounding of the tissue. TGF-β, transforming growth factor-β.

Results obtained using this model yielded a 50% increase in the density of blood vessels in both hypertrophic and keloid scarring phenotypes, which is expected from experimental observations. Their model predictions also had therapeutic implications for the treatment of keloids, namely that collagen levels could return to normal if an inhibitor of NO (such as L-NMMA) was used, while the excess scar tissue was surgically removed.

THE INFLUENCE OF MATHEMATICAL MODELING ON BIOLOGICAL EXPERIMENTS

Mathematical models have been used to provide insight not only into the wound healing process and the healing under treatments of wounds but also into biological laboratory experiments that are frequently used in the context of wound healing, two of which are discussed below.

Wound healing assays

There have been several experiments designed to investigate aspects of collective cell migration during epidermal wound closure. One of the best-known techniques designed to study this behavior is the wound healing assay, or *in vitro* scratch assay.[104] As illustrated in Fig. 9, this involves making a "scratch" in a confluent monolayer of cells, such as keratinocytes, in a tissue culture dish, to create an artificial wound. Over time, the cells begin to close this gap through the process of migration. In addition to being a highly versatile, and easy to prepare, experimental setup, it allows for tracking the motion of individual cells during the process of closure.

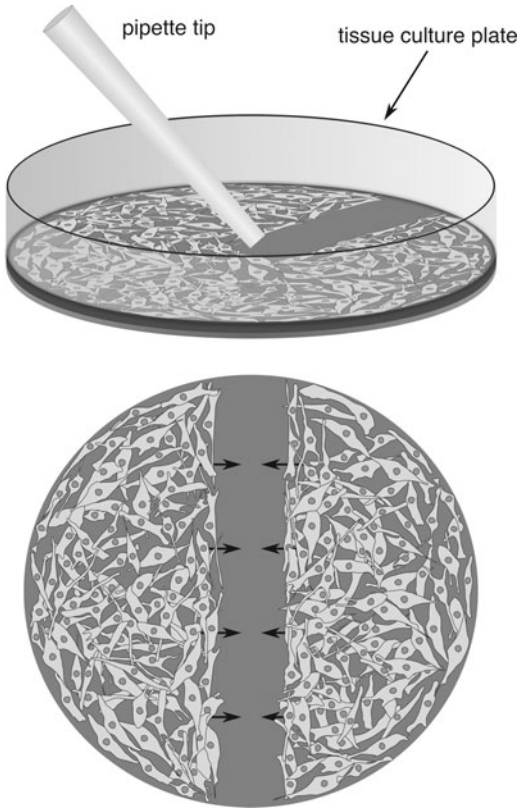

Figure 9. Schematic illustration of a wound healing assay. Cells are placed in a tissue culture dish and are allowed to develop a confluent monolayer. Subsequently, a scratch in the layer is created using a narrow object such as a micropipette tip. The closure of this scratch can then be examined using different techniques, including bright-field microscopy.

One of the first mathematical models of the migration of cells in a wound healing assay was developed by Maini *et al.*[105] They assumed that the two most significant physical processes governing the behavior of cells in such colonies are migration through random diffusion and growth of the population through cell proliferation, allowing them to model the invading front of cells. Despite the simplicity of this model, they observed that it was consistent with experimental observations where, after an initial duration, the cells at the edge of the front moved at a roughly constant speed.

Subsequently, Cai *et al.* developed a pair of models for the collective migration of cells in a wound healing assay.[106] In contrast to earlier approaches, they developed a continuum model in which migration was mediated by contact inhibition, and hence cell diffusivity depended locally on cell density. They then derived a discrete model in which individual cells were described as continuous-time random walkers that exhibited nearest-neighbor transitions,

as well as births and deaths. Comparing results obtained from simulations of their continuous-time random walkers with those using their continuum model revealed that the latter is insufficient to describe certain experimental observations, for instance that cells have a bias toward regions of lower density.

Due to the relative simplicity of the experimental setup, many studies have attempted to extract information regarding the cell diffusivity and rate of proliferation by tracking the leading edge of cells in a scratch assay. However, as Johnston *et al.* demonstrated, it is not straightforward to unambiguously estimate these values from observed data.[107] To show this, they developed a lattice-based random walk model, in which individual cells have the ability to move to neighboring sites or proliferate (and thereby place a daughter cell at a neighboring site). Comparing results obtained through this model with those from a scratch assay experiment, they found that a close fit to the data could be obtained for a wide range of choices of cell diffusivities and proliferation rates. However, upon separating the experimental data into two time intervals, they observed that if the cell diffusivity was estimated from the initial interval, and the cell proliferation from the latter, the resulting values were consistent with previously reported observations.

While several of the models proposed to describe the collective migration of cells during wound closure are either of the reaction-diffusion type, or follow discrete cell-based approaches, alternative modeling frameworks have also been employed. For example, Arciero *et al.* described the layer of cells as a 2D compressible fluid, and developed a model for the evolution of the density of cells, which accounted for the force of adhesion of the layer to the underlying substrate and the various stresses within the layer.[108] They observed that simulations of their model closely agreed with experimental observations for the shape of a shrinking "gap" in a wound healing assay, as well as the corresponding cell densities.

Fibroblast-populated collagen lattices

As the regulation of the remodeling process is crucially dependent on the stresses that cells in the wound, as well as the ECM, experience from the surrounding (healthy) tissue, it remains a challenge to design *in vitro* experiments with settings that accurately replicate the mechanochemical environment that the cells and ECM are embedded in. One of the earliest attempts at investigating the behavior of fibroblast cells in such a setting was the development of fibroblast-populated collagen lattices (FPCLs) by Elsdale and Bard,[109] which consisted of cultured fibroblasts that were embedded within, or on top of, three-dimensional collagen matrices. Subsequently, there have been numerous experiments performed on FPCLs to investigate the traction forces that fibroblasts exert in both mechanically relaxed[110–112] as well as mechanically loaded environments,[113–115] and how the activity of fibroblasts in such environments is mediated through various growth factors.[116,117] See Refs. 118–120 for detailed reviews of experiments performed on FPCLs.

The fibroblasts in such gels reorganize the fibers of the collagen matrix along the direction in which they spread.[113,121,122] The change in the mechanical structure of FPCLs leads to its rapid contraction (within a few days) to a small fraction of their initial size.[110] This contraction has been found to be permanent,[113,121,122] that is, even if gel reorganization is suppressed, for instance, by adding cytochalasin D, one only observes a partial reexpansion of the FPCL. In other words, the activity of fibroblasts leads to an irreversible mechanical restructuring of the collagen matrix, a crucial process that is also seen *in vivo* during the latter stages of wound healing.

There are three broad classes of FPCLs: free floating, attached and stress relaxed, which are classified based on the mechanical environment they are embedded in (for a detailed discussion of the different types of FPCLs, as well as an overview of the attempts to mathematically model their contraction, see Menon *et al.*[71]). As illustrated in Fig. 10, the different classes of FPCLs exhibit distinct types of contraction with, for example, free-floating FPCLs reducing in both height and radius and attached FPCLs reducing in height alone. The degradation and replacement of collagen in attached lattices are not significant factors in their eventual contraction,[113] which suggests more generally that the rearrangement of collagen fibers by fibroblasts is the prime mechanism of contraction in FPCLs.

While there have been attempts to mathematically model the contraction of FPCLs using a viscoelastic framework with a Kelvin-Voigt constitutive law,[123] or a Maxwell constitutive law,[124] these were only valid under very specific circumstances and could not account for some aspects of FPCL contraction. Although there have been several subsequent attempts at modeling the contraction of FPCLs,[125–132] these models do not take into account the mechanical restructuring of the FPCL that continually occurs during the processes of re-

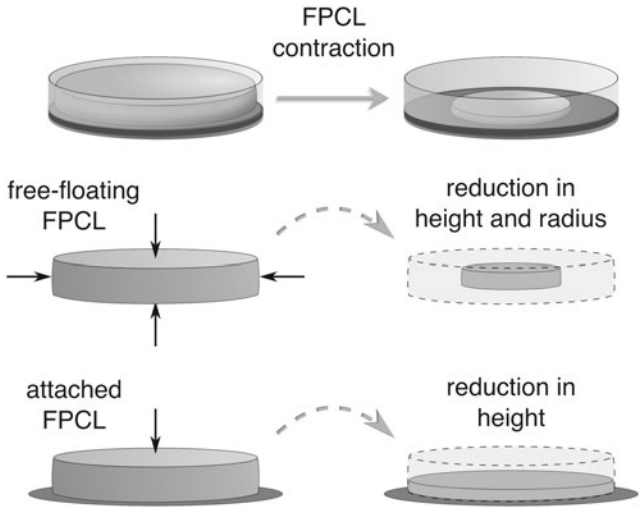

Figure 10. Schematic illustration of the contraction of an FPCL. The nature of contraction varies depending on the type of FPCL under consideration. Free-floating FPCLs exhibit a reduction in height as well as diameter, while attached FPCLs adhere to the base of the tissue culture dish that they are polymerized in, and exhibit a decrease in height, but not in lateral area. FPCL, fibroblast-populated collagen lattice.

modeling and contraction. The model of Menon *et al.*[71] attempted to address this by introducing an equation for the evolution of the "effective strain,"[133] which provides a measure of the difference between the current state of the system and its zero-stress state (which evolves over time). As the contraction of such lattices primarily occur over a single spatial dimension, they developed a one-dimensional model for FPCL contraction, consisting of a pair of coupled ODEs for the time evolution of the displacement gradient and effective strain of a gel. Simulations obtained using this model captured experimental observations for different types of contracting FPCLs.

Future challenges

As the process of wound healing is a tightly orchestrated and highly complex sequence involving a wide array of participating cells, growth factors, and other components, perhaps unsurprisingly, there remain a large number of open questions in the field. For instance, two aspects of wound healing that would benefit from further investigation using mathematical modeling are the process of vessel regression during remodeling, and proteolysis, in which matrix metalloproteases degrade the basement membrane of the blood vessels, allowing ECs to escape their parent vessel. Furthermore, modeling may help address questions associated with the transition between the proliferation and remodeling stages of healing, such as the feedback mechanisms responsible for shutting the healing process down, including regression of newly formed vessels when no longer required.[134]

The interpretation of model results in light of clinical/experimental observations (Fig. 1) requires appropriate parameterization, and the predictive capacity of any mathematical model is reliant on appropriate values of the model parameters. To date, statistical methods have not been routinely adopted for parameter estimation in the field of mathematical models of wound healing, as has been the case in other fields of mathematical biology, such as infectious disease modeling and mathematical ecology.[135,136] There is therefore significant scope for applying modern applied statistical techniques to rigorously infer parameter values in models of wound healing, especially given the various types of data that are available in the literature (animal models, *in vitro,* etc.). In this regard, experiments on scratch assays (discussed in Wound Healing Assays section) have facilitated the estimation of wound healing model parameters.[137]

Another key challenge associated with the use of mathematical models to describe wound healing is the issue of parameter identifiability, a property of a model that must be satisfied in order for precise parameter inference to be possible.[138,139] That is, given a mechanistic model and an observation process, there is no guarantee that the model parameters are able to be estimated regardless of how much data are collected. Even if a model is identifiable from a theoretical point of view, there may still be significant parameter estimation issues that arise from the quality and quantity of the data available.

To date, mathematical models have been developed to assess treatment strategies, including HBOT and alternative designs for compression bandages.[87,89,90] However, there remain several open questions, including how negative pressure and electric fields can assist the treatment of wounds. Treatments at the cellular scale in wounds can also benefit from further theoretical studies, including the role of immune cells in regulating angiogenesis, and the mechanisms through which vascular endothelial growth factor or Delta-Notch signaling pathways promote or inhibit angiogenesis.[140,141] Modeling can also help probe the efficacy of the treatment of chronic wounds with cultured skin substitutes, which replenish the ECM in the damaged tissue.[142–144] Finally, models of sufficient complexity could account for variations in the wound environment, as well as treatment regimes, and make predictions that could assist in the development of individualized treatment strategies.

SUMMARY

In this article, we have presented an overview of the diverse ways in which mathematical modeling can provide deep insights into the mechanisms that underlie aspects of wound healing, covering seminal works over the last few decades as well as recent advances in the area. We have highlighted the contributions such models have made toward the understanding of the interplay between the array of components that underlie the healing process, and also to the development of improved treatment strategies. Furthermore, wound healing models can draw from advances in tumor modeling, as there are several similarities between aspects of the two processes, most notably, angiogenesis.[29] In recent years, biological data have become available in unprecedented quantities and at fine-scale spatiotemporal resolutions. As such, there is a current need for the development of novel mathematical models and statistical inference methods that can maximize the utility of the data. Tackling this problem will require an interdisciplinary team of researchers with a broader set of skills, including the numerical solution of mathematical models, software development, and statistical inference for model parameter inference. Such a team may comprise, for instance, biologists, applied mathematicians, clinicians, physicists, computer scientists, and applied statisticians. Interdisciplinary collaborations will be essential to deal with the large amounts of data being collected and the complex *in silico* mathematical models available. Furthermore, research students require sufficient exposure to both modeling and experimental techniques. Looking ahead, the development of a common language for interdisciplinary wound healing research would be essential. This could potentially be achieved by (i) disaggregating the underlying biological processes in terms of the physical mechanisms associated with the constituent components such as cells and tissues and (ii) interpreting model predictions in terms of experimentally measurable quantities. This would help sustain a robust dialogue between collaborators from experimental and theoretical backgrounds, without requiring joint expertise in both areas. We hope that this review can contribute to these efforts by highlighting the contributions of wound healing models to date.

TAKE-HOME MESSAGES

- Mathematical modeling can help provide insight into aspects of wound healing, which would otherwise require difficult and costly experiments to investigate.
- The key benefit of the modeling approach is that it facilitates the identification of those components that contribute most significantly to the process under consideration.
- Over the last few decades, mathematical models have enhanced our understanding of the different phases of healing, and have also helped probe the efficacy of a range of treatment strategies.
- Such mathematical models may yield testable predictions that can stimulate focused experimental research on the roles played by different biological components during wound healing.
- With the increasing availability of wound-related data at fine-scale spatiotemporal resolutions, interdisciplinary collaborations will be essential to advance this field of research.

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ABOUT THE AUTHORS

Shakti N. Menon, PhD, received his PhD in Applied Mathematics from the University of Sydney, and is currently a Project Scientific Officer at the Institute of Mathematical Sciences, Chennai. His research interests include collective behavior, and self-organization in complex systems. **Jennifer A. Flegg, PhD,** received her PhD in Applied Mathematics from the Queensland University of Technology in 2009, and is currently an Associate Professor at the University of Melbourne, Australia. Her research interests are in mathematical biology, including wound healing, tumor growth, and infectious diseases.

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Abbreviations and Acronyms

2D = two dimensional
EC = endothelial cell
ECM = extracellular matrix
EGF = epidermal growth factor
FPCL = fibroblast-populated collagen lattice
HBOT = hyperbaric oxygen therapy
KGF = keratinocyte growth factor
NO = nitric oxide
ODE = ordinary differential equation
PDGF = platelet-derived growth factor
TGF-β = transforming growth factor-β