

Systems-based approaches toward wound healing

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Wound healing in the pediatric patient is of utmost clinical and social importance because hypertrophic scarring can have aesthetic and psychological sequelae, from early childhood to late adolescence. Wound healing is a well-orchestrated reparative response affecting the damaged tissue at the cellular, tissue, organ, and system scales. Although tremendous progress has been made toward understanding wound healing at the individual temporal and spatial scales, its effects across the scales remain severely understudied and poorly understood. Here, we discuss the critical need for systems-based computational modeling of wound healing across the scales, from short-term to long-term and from small to large. We illustrate the state of the art in systems modeling by means of three key signaling mechanisms: oxygen tension-regulating angiogenesis and revascularization; transforming growth factor- β (TGF- β) kinetics controlling collagen deposition; and mechanical stretch stimulating cellular mitosis and extracellular matrix (ECM) remodeling. The complex network of biochemical and biomechanical signaling mechanisms and the multiscale character of the healing process make systems modeling an integral tool in exploring personalized strategies for wound repair. A better mechanistic understanding of wound healing in the pediatric patient could open new avenues in treating children with skin disorders such as birth defects, skin cancer, wounds, and burn injuries.

Dermal wound healing in the pediatric patient is a symphony of events precisely synchronized to repair the damaged tissue, restore its protective barrier function, and safely return it to its homeostatic equilibrium state (1). Although the underlying processes, cell-matrix interaction, cell-cell cross-talk, and cellular mechanotransduction, involve a complex cascade of events, dermal wound healing is robust and rarely diverges to malignant transformation (2). Yet it is not always perfect. Prenatal skin usually heals smoothly to seamlessly restore the state before injury (3), whereas postnatal skin is incapable of healing wounds tracelessly, leaving scar behind. Postnatal skin can easily restore its protective barrier function; however, the resulting scar rarely has the same microstructure, collagen content, and mechanical properties as the native tissue (4). In extreme cases, pronounced fibrotic activity might even initiate hypertrophic scarring, characterized by an excessive collagen deposition (5). In the pediatric patient, excessive scarring has consequences throughout early childhood and

adolescence and can lead to low self-esteem or even stigmatization (6). The prevalence of hypertrophic scarring in the pediatric population is overwhelming; of the total cases of burn scars and keloids, 70% occur in children (7).

The underlying mechanisms of scar formation are now better understood than ever before, and we have made tremendous progress toward improving and accelerating healing mechanisms (8). We have come to appreciate that the healing process spans various temporal and spatial scales and is affected by both chemical and mechanical cues. However, even with the detailed insight that traditional approaches have provided on the individual scales, the behavior of the system as a whole remains elusive. Computational modeling is increasingly recognized as a powerful tool to provide insight into the dynamics of wound healing and the interaction of biochemical and biomechanical phenomena across the different scales (9).

Fortunately, dermal wound healing, like all inflammation-based processes in the human body, is based on various redundant signals and cross-talk between different signaling networks (10). Although redundancy is hugely beneficial for the biological system itself, it complicates the overall understanding of the healing process: even if individual elements of the signaling network are well understood in isolation, the coupling of these elements is hugely complex, and it is virtually impossible to gain basic insights based on sparse experimental data. Computational systems biology is of acknowledged importance in advancing our holistic understanding of pediatric wound healing: in the short term, computational systems biology allows us to systematically explore controlled what-if scenarios and virtually probe various hypotheses to better understand the healing process as a whole (11); in the long term, given the incredible variability of healing responses between different individuals, computational systems biology is an integral ingredient to shape the future of personalized medicine (12).

TEMPORAL SPECTRUM OF WOUND HEALING

From the moment of injury until the tissue reaches its final configuration in the form of a mature scar, months or even years may elapse. However, the protective function of skin has to resume immediately to avoid dehydration, infection, and loss of tissue integrity. Accordingly, the initial phase of healing takes only a few minutes. The process of wound healing is commonly divided into four overlapping phases: hemostasis,

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inflammation, proliferation, and remodeling (see **Figure 1**, rows 1 through 4).

During Hemostasis, the Injured Region Fills With a Platelet-Rich Fibrin Clot

Hemostasis occurs in the order of minutes. During hemostasis, the wounded space rapidly fills with a clot to stop blood loss and to re-establish a barrier from the outside world. In the later phases of healing, this clot will serve as a temporary matrix for the cells that migrate toward the wound to reconstruct the dermal tissue (**Figure 1; 1c**) (13). The clot is composed mainly of fibrin fibers and platelets. The cytoplasm of the platelets carries α -granules from which a cocktail of growth factors and cytokines is released when the cells degranulate (**Figure 1; 1b**) (14). Proinflammatory signals from the platelets are directly reflected in molecular changes in the endothelial cells of the blood vessels near the injury site. A chemotactic

response attracts leukocytes such as neutrophils and monocytes (**Figure 1; 1a**), which sense these molecular changes and adhere to the endothelial cells. The ongoing chemotactic pathway and the interaction between leukocytes and endothelial cells lead to the capture and transmigration of the neutrophils and monocytes in a process called diapedesis (15).

During Inflammation, Leukocytes Establish Chemotactic Gradients

Neutrophil diapedesis marks the beginning of the inflammatory phase. Neutrophil transmigration also occurs very rapidly; in fact, the first neutrophils reach the wound within a few minutes, but they keep being recruited for the next 2 or 3 d. The primary function of neutrophils is to dispose of pathogens entrapped in the clot at the moment of injury. Their secondary function is to amplify the proinflammatory indicators. Monocytes arrive at the wound within 2 d of the initial insult and differentiate into

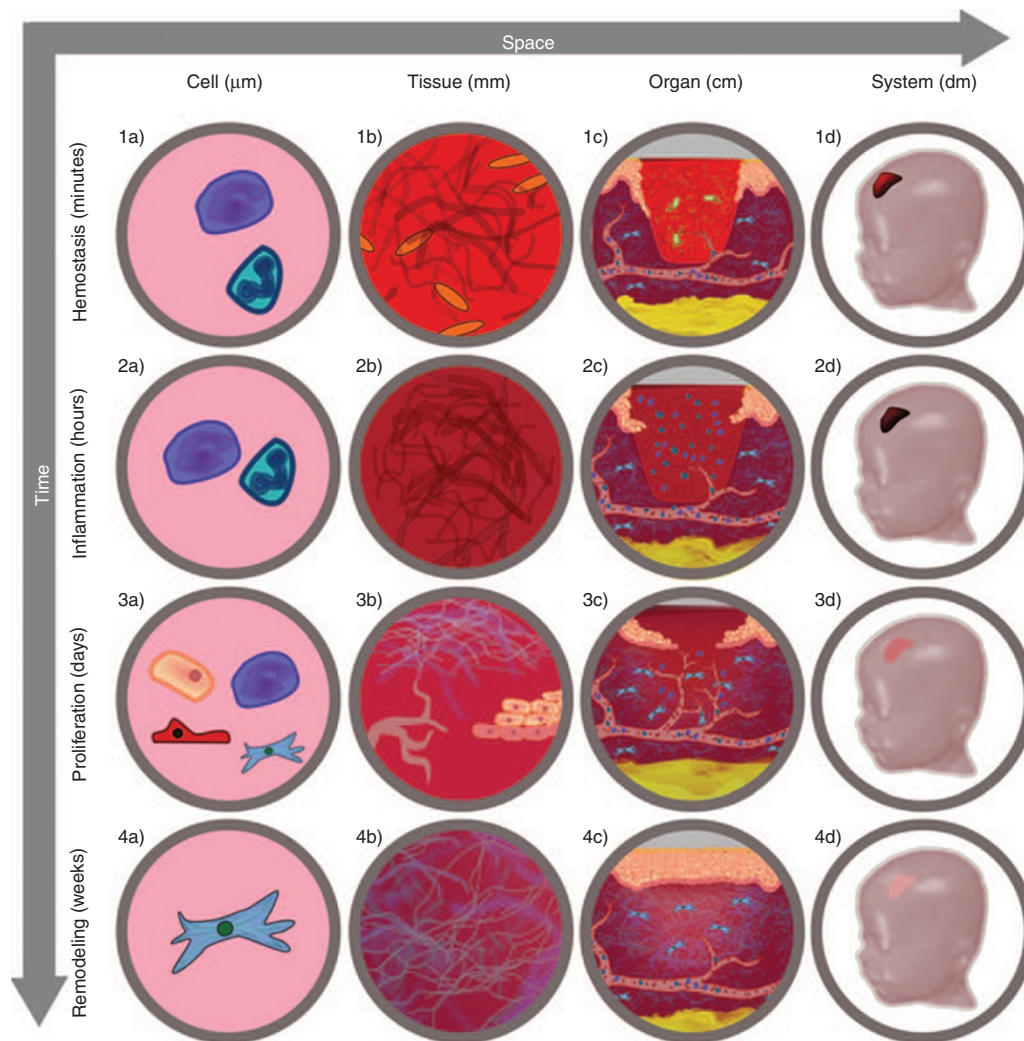


Figure 1. Spatiotemporal spectrum of wound healing. Wound healing is a hierarchically orchestrated process that spans interacting phenomena from minutes to months, from the cellular to the system level. Temporally, homeostasis takes place within the first minutes (row 1), inflammation within hours (row 2), proliferation within days (row 3), and remodeling within weeks to months (row 4). Spatially, the cellular level is associated with length scales in the order of micrometers (column a), the tissue level with millimeters (column b), the organ level with centimeters (column c), and the system level with decimeters (column d).

macrophages (Figure 1; 2a). The role of monocytes is twofold: they phagocytose the remaining matrix debris, pathogens, and neutrophils, and they produce soluble chemical mediators such as transforming growth factor- β (TGF- β) and vascular endothelial growth factor. Both TGF- β and vascular endothelial growth factor are crucial to guiding the recruitment of fibroblasts and endothelial cells later in the healing process (Figure 1; 2c).

During Proliferation, Fibroblasts Deposit Collagen and New Vasculature Forms

During the proliferative phase, two processes occur simultaneously: re-epithelialization and replacement of the fibrin clot by granulation tissue (Figure 1; 3c). These steps in the repair process continue throughout 2–3 wk. In this phase, the sharp chemoattractant gradients generated through the inflammatory phase attract different cell types to manufacture new tissue (Figure 1; 3a). At the epidermal level, keratinocytes in the wound edge dissolve their adhesions to the basal lamina in order to crawl and reproduce on top of the fibrin matrix, rebuilding the epidermis over the injured region (Figure 1; 3b). On the dermal level, fibroblasts are in charge of depositing collagen, the main load-bearing constituent of skin (Figure 1; 3b). The metabolic needs resulting from increased cellular proliferation and migration require the formation of a vast network of capillary tubes to provide nutrients and oxygen. Endothelial cells rapidly form a new vasculature in a process referred to as angiogenesis (Figure 1; 3b). Toward the end of this phase, some fibroblasts transform into myofibroblasts, which actively pull on the wound edges to contract the injured tissue.

During Remodeling, Fibroblasts Gradually Restore Skin Integrity and Homeostasis

Once the fibrin matrix has been replaced by granulation tissue made of fibroblasts, the remaining macrophages, and a vascular network, the remodeling phase begins. At this point, the wound is fully closed, yet the tissue has very poor quality. It consists primarily of thick, aligned collagen bundles, instead of the interwoven collagen networks found in native skin (Figure 1; 4b). Dynamic changes continue, but on a much slower time scale. Remodeling can go on for months or even years. Finally, the vascular network retracts and most of the cells undergo apoptosis or migrate out of the affected region. The remaining fibroblasts (Figure 1; 4a) gradually restore skin integrity and mechanical homeostasis (Figure 1; 4c). However, unlike prenatal skin, postnatal skin usually never fully recovers its original native state.

SPATIAL SPECTRUM OF WOUND HEALING

Similar to virtually all biological phenomena, in wound healing, temporally and spatially interacting events at the molecular, sub-cellular, cellular, tissue, and organ levels ultimately converge to a well-defined system-level response (16) (see Figure 1, columns a through d). This macroscopic response is inherently rooted in the hierarchical structure, which is established through a precisely defined biological organization (17). Although it is in principle possible to zoom in to the subcellular or even molecular scales, the cellular level is the preferred starting point in the context of

wound healing modeling, mainly because cells are the smallest autonomous building blocks in the biological hierarchy (18). One level above in the organization, at the tissue level, we find interacting cell aggregates embedded in the extracellular matrix (ECM). The next larger scale corresponds to the organ level, the level of the wound itself. In principle, we could even contemplate higher levels of organization, for example, entire organ systems. Here, however, we do not consider larger spatial scales, because all relevant phenomena take place within the local wound environment.

At the Cellular Level, Chemomechanical Stimuli Guide Cellular Decision Making

The smallest scale we explore here is the cellular level, a scale in the order of 10 μm . At this scale, four cell types are critical to wound healing: endothelial cells, fibroblasts, macrophages, and keratinocytes (Figure 1; 1a–4a). For each cell type, there are specific regulatory mechanisms that are key to a successful repair process. The most important control mechanism is chemotaxis, the migration of cells in the direction of increasing chemical gradients (19). Endothelial cells, fibroblasts, and leukocytes migrate into the wound site attracted by the strong concentration of different growth factors and other chemical mediators released during the inflammatory phase (20–22). Chemotaxis in eukaryotic cells is complex and, unlike for prokaryotic systems, there are no established models to predict how eukaryotic cells migrate as a function of chemical gradients. Prokaryotic cell migration resembles a biased random walk, which can be easily represented by simple mathematical models. Eukaryotic cells, however, move in continuous paths with smooth turns toward ascending signal concentrations but display an overall stochastic behavior (23–25).

In addition to chemical cues, mechanical cues play a significant role in wound healing (26,27). The ultimate goal of skin repair is to re-establish the mechanical load-bearing capacity to restore the homeostatic equilibrium state. Key to this restoration is the translation of mechanical signals into chemical reactions, a process known as mechanotransduction, which is the landmark characteristic of fibroblasts in the repair sequence (Figure 1; 4a) (28). Fibroblasts anchor to the ECM through focal adhesions. These adhesion sites are linked to stretch-activated ion channels across the cell membrane and to the cytoskeleton inside the cell. Altering the mechanical scenario of the ECM directly creates a flow of charged ions via these ion channels and indirectly governs cellular behavior through perturbed cytoskeletal dynamics (29). Fibroblasts respond to mechanical changes in the ECM in several ways: by depositing collagen, decreasing apoptosis rates, increasing inflammatory signals, and transforming into myofibroblasts to actively pull on the wound edges (30–32).

For endothelial cells and keratinocytes (Figure 1; 3a), cell-cell and cell-matrix adhesions are of primary importance throughout the entire repair process. Keratinocytes critically depend on cell-cell and cell-matrix cross-talk to define their position in the epidermal lattice. The polarization of keratinocytes directs lateral migration and proliferation during re-epithelialization (33). Endothelial cells also depend on these types of interaction during angiogenesis (34). Some cells in the tip of sprouting vessels are activated and protrude filopodial

extensions, which actively interact with their immediate micro-environment to guide angiogenesis. The remaining endothelial cells support the leading tip by maintaining the connectivity with the parent vessels and by helping in the maturation of the newly formed capillary tubes (35).

At the Tissue Level, Chemomechanical Fields Coordinate Cellular Organization

On the next level of the hierarchy, we are interested in the bulk mechanical properties of the ECM, the diffusion of growth factors and the overall behavior of cellular aggregates (Figure 1; 1b–4b). The properties of the ECM and the diffusion of growth factors are crucial at this scale, because they contribute a key aspect of the healing process: they pass information across distances larger than the characteristic cell size, coordinating the action of multiple cells without using direct cell–cell communication. Soluble mediators diffuse chemical cues, and the ECM transmits mechanical cues across the tissue (Figure 1; 1b and 2b). To characterize chemical diffusion in an isotropic medium, only a single constant needs to be specified. To characterize mechanical signaling of the ECM, however, we can select from a huge variety of constitutive models, and two or even more constants need to be determined (Figure 1; 4b) (36–38).

Two other phenomena that become important at the tissue level are pattern formation and collective cell migration. An illustrative example is re-epithelialization, associated with the formation of an advancing front that has distinct collective features entirely different from those of the individual cellular response. The leading edge of keratinocytes that invade the wound adopts a wave propagation profile, which can be characterized using Fisher's equation (Figure 1; 3b) (39). Another even more intriguing proof of complex organization is angiogenesis, a highly coordinated process that cannot be understood solely from the study of a single cell. During angiogenesis, a new vasculature forms from existing blood vessels that surround the injured region (40). This process consists of combined tip chemotactic diffusion and sprouting to create a new network of capillary tubes (Figure 1; 3b) (41).

At the Organ Level, the Chemomechanical Environment Controls Wound Healing

The final level of interest in wound healing is the spatial region that contains the injured skin and its immediately surrounding healthy tissue (Figure 1; 1c–4c). At this level, we witness the healing capacities of skin as the result of a perfectly organized interaction of all phenomena described above: from micro-structural decisions of the individual cells, via mesostructural properties of the cells embedded in the ECM, to macrostructural reaction–diffusion systems of the soluble mediators and the patterning capabilities of cellular aggregates.

OVERVIEW OF COMPUTATIONAL MODELING FOR SYSTEMS BIOLOGY

Mathematical representations can foster our understanding of biological systems. An abstraction of a biological system consists of characteristic variables, such as the concentration of a

chemical substance or the cell density in a region of tissue, and the interaction rules between the different components of the system. Mathematical models are representations of the real world and, as such, they can be created in many different ways depending on the features of the problem itself, on the information at hand, and on the mathematical tools preferred by the modeler. Creating a mathematical model is only the first step of a simulation process. The second step consists of converting the mathematical model into a computational model that allows solving complex patient-specific problems.

Mathematical Models Are Sets of Equations That Characterize the Dynamics of a Biological System

In the context of wound healing, there are two conceptually different mathematical approaches to representing a biological system: continuum models and discrete models. In the physical world, substances can vary smoothly over a given domain. In this case, we represent them through a function called a continuous field. Continuous fields are the basic representation of continuum models. Typical continuous fields in wound healing are concentrations of chemical mediators such as TGF- β . Some components of the system may be large enough to be considered as discrete entities. To model individual entities, we typically use discrete models that represent the individual components explicitly and characterize their interaction through simple mathematical equations. Typical discrete entities in wound healing are cell populations such as densities of fibroblasts and macrophages. The choice between continuum and discrete models strongly depends the particular scale of interest. Components that can be considered as discrete entities at the molecular and cellular level become averaged out at the tissue and organ level, at which they can be approximated as continuous fields. In general, the scale of interest will determine which modeling approach is more appropriate.

In systems biology, different types of interacting components have to be represented through the mathematical model, either as continuous fields or as discrete entities. A multifield model is one that consists of several interacting fields. Typical multifield phenomena in wound healing models are various interacting chemical signaling pathways, such as the TGF- β and vascular endothelial growth factor pathways. In some cases, it might be advantageous to represent some variables as continuous fields whereas others might be represented as discrete entities. Accordingly, there are three basic types of representations: purely continuum, purely discrete, and hybrid continuum/discrete. In addition, a mathematical model for each of these representations can span various spatial scales, from the cellular level, via the tissue and organ levels, to the system level. We characterize these types of models as multiscale models.

From a modeling point of view, biological systems consist of three basic ingredients: chemical substances, cell populations, and mechanical factors. Chemical substances are generally treated as continuous fields. Cell populations can either be modeled individually or as fields, depending on the scale of interest. Here, we use the notion of biological field for fields that represent cell populations. Mechanical factors include forces and deformations and are almost always treated as continuous fields.

In continuum models, the evolution of different fields in space and time is governed by partial differential equations. A partial differential equation establishes (i) how a field interacts with other fields through coupling terms; (ii) how a field evolves in time; and (iii) how a field varies in space over the domain of interest. One partial differential equation is needed for each field. Continuum models represented through partial differential equations are an elegant and compact way to characterize spatial variations of chemical concentrations, mechanical strains, or mechanical stresses.

In discrete models, the behavior of each entity is governed by one or more ordinary differential equations. An ordinary differential equation establishes (i) how a characteristic variable of a single entity interacts with other variables through coupling terms; and (ii) how a characteristic variable evolves in time.

Computational Models Are Algorithmic Realizations of a Mathematical Model

In pediatric wound healing, the mathematical model is typically too complex to be solved analytically. Through a process called *discretization*, we can transfer the *mathematical model*, the set of equations, into a *computational model*, an algorithmic solution of these equations, that can easily simulate phenomena of various degrees of complexity.

Continuum models are typically represented through coupled partial differential equations. To solve the underlying set of equations, it is necessary to subdivide or discretize both space and time. Time is almost always discretized into small intervals by means of the finite difference methods. Discretizing space can be performed in several ways: the simplest approach to represent the domain of interest is a regular mesh, as is used in the finite difference method and the finite volume method. To represent arbitrarily shaped domains such as particular parts of the body, the preferred approach is the use of the finite element method. This method approximates the domain by subdividing it into little pieces called elements. Although finite elements are geometrically more versatile than finite differences and finite volumes, their algorithms are more sophisticated and require special care of implementation.

Once time and space are discretized, we obtain a system of algebraic equations. The solution of this system characterizes the evolution of all continuous fields, both in space and time. Depending on the required level of accuracy, the solution procedure can become a challenging and computationally expensive task. Consequently, we can select between various useful simplifications: In the real world, the domain of interest is three dimensional. However, we can often extract useful information about the biological system by approximating it as one or two dimensional. Another simplification could be a particular symmetry of the system. Typical examples are cylindrical symmetries, which can be approximated by one-dimensional formulation referred to as axisymmetric.

Discrete models are typically represented through ordinary differential equations. These equations do not need to be solved over the entire domain; they are solved locally to predict the evolution of each individual entity in time. Because only

the time needs to be partitioned into small intervals, and not the space, it is common to use the finite difference method. Although discrete models are conceptually simpler than continuum models, they are computationally more expensive because every single entity has to be represented through its own equation. When dealing with entire cell populations, their computational cost becomes overwhelming.

GUIDELINES

From the above discussion on the basic approaches toward systems-based mathematical representations, and to establish a framework for a guided discussion of the most recent wound healing models, we propose a few useful guidelines for effective computational modeling of wound healing.

Simplified One-Dimensional and Axisymmetric Models Can Provide Quick Insight

One-dimensional and axisymmetric models provide a quick first insight into the interplay of individual signaling pathways during wound healing. They are of great value for quickly testing therapeutic hypotheses. Because of their conceptual simplicity, one-dimensional and axisymmetric models should be viewed as a validated and well-calibrated starting point for further model refinement. For detailed analyses that focus on the spatiotemporal interaction of signaling pathways in wound healing, however, we recommend using two- or three-dimensional models.

Discrete Models Can Answer Fundamental Questions at the Molecular and Cellular Levels

We recommend using discrete models to study the biochemistry of individual cells. Discrete models are typically based on simple relations and straightforward to implement. Because they are computationally expensive, however, they are only feasible for answering fundamental scientific questions at small scales. We recommend fully avoiding discrete models when diffusion or mechanical cues play an important role.

Continuum Models Can Answer Fundamental Questions at the Tissue, Organ, and System Levels

We recommend using continuum models to study the interaction of biochemical and biomechanical fields. Continuum models are computationally efficient but require special care when translating the collective action of individual cells to a continuous field. When embedded in efficient finite element solvers, continuum models allow for a patient-specific analysis based on individualized, realistic three-dimensional geometries. Continuum models are ideal to answer translational clinical questions at larger scales.

Multiple-Field Models Provide Insight Into the Cross-Talk Between Chemistry, Biology, and Mechanics

Although models focusing on a single individual aspect of wound healing can provide specific insight, holistic models are currently becoming the gold standard in wound healing simulations. By contrast to single-field models, holistic multifield models can reliably represent the cross-signaling

networks during wound healing. When creating a multifield model, we recommend including a representation of at least five interacting fields: (i) fibroblast population; (ii) endothelial cell population and/or nutrient concentration; (iii) indicator of ECM restoration such as collagen content; (iv) chemoattractant concentration; and (v) inflammatory cells population. This baseline five-field model can, of course, be further refined if specific aspects of the healing process are of interest.

SYSTEMS-BASED MATHEMATICAL MODELS OF WOUND HEALING

Over the past two decades, mathematical and computational modeling have advanced as key players in the quest for understanding the complex multiscale phenomena involved in wound healing. Ideally, once calibrated and validated, these models can serve as tools to quantify the impact of selected perturbations to the baseline system and, ultimately, to predict the effect of different therapeutic treatment options.

In an effort to gain basic scientific insight into the process of wound healing, researchers in the early '90s started to develop mathematical models of the repair response of skin. Sherratt and Murray are often considered to be the fathers of modern mathematical modeling of wound healing (42). Since their first model was published more than two decades ago, a multitude of modifications, improvements, and enhancements have been proposed. The characteristics, the focus, and the simplifications of each model are different, based on the fundamental question it seeks to answer. This makes it challenging to define a unique benchmark for a scientist or clinician who would like to make use of the existing models or propose a new one.

It is becoming clear, however, that the current trend in computational systems biology converges toward multiscale, multifield modeling. In the time domain, most analyses focus on shorttime scales, mainly on the inflammatory and proliferative phases, and only a few more recent models integrate larger times scales including the remodeling period (43). In the space domain, modelers are now giving priority to multiscale integration, from a basic mathematical representation of the cell to fully three-dimensional models of the wound site.

Here, we suggest a classification of existing models based on the aspect of wound healing they seek to address and on the mathematical tools they use to derive their governing equations, see **Table 1**. In this classification, the three major focus areas are re-epithelialization and cell migration; angiogenesis; and wound contraction and collagen deposition. The four

degrees of modeling complexity are one-dimensional or axisymmetric continuum models; two-dimensional continuum models; two- or three-dimensional discrete models; and two-dimensional hybrid discrete/continuum approaches.

We hope this classification alongside the guidelines discussed above will provide new computational scientists and clinicians with the necessary framework to make use of the existing models or to create improved versions attending to the specific question they want to answer.

To better illustrate which modeling approaches have been used and how they have enhanced the understanding of wound healing from the perspective of systems biology, the following subsections present, for each major focus area defined in **Table 1**, the modeling and solution methods that have proven to be more effective, followed by an example of how computational simulations have tested virtual hypotheses by altering the baseline model and analyzing the outcome of the computations.

Re-epithelialization Creates Propagation Patterns of Constant Velocity Waves

The simplest mathematical models in wound healing focus on re-epithelialization and cell migration. These models typically characterize a cell population over a domain in terms of point-wise cell densities rather than considering each individual cell. Fisher's equation is the preferred representation of the population behavior. It consists of a diffusion term to capture cell migration and a logistic term to capture cell proliferation (39). This abstraction is well suited for the study of re-epithelialization and is also considered useful for fibroblast and endothelial cell migration in wound healing (39,44). During re-epithelialization, keratinocytes remain in stretch contact with one another, maintaining tissue continuity throughout the epidermis. A continuum assumption is therefore well justified. The solution to Fisher's equation is a wave propagation pattern in which the advancing front of cells moves with an almost constant velocity (44). Although the model is conceptually two dimensional (45,46), most numerical implementations are based on the additional assumption of axisymmetry to further simplify the modeling. A more sophisticated alternative to these continuum models is the discrete model, which represents each individual cell explicitly (47). Discrete models typically describe the motion of each cell as a reinforced random walk in the direction of a stimulus and regulate cell division through an internal clock (48).

Table 1. Systematic classification of mathematical models for wound healing based on model complexity and focus area

Complexity/focus	Reference citation number		
	Re-epithelialization and cell migration	Angiogenesis	Wound contraction and collagen deposition
One-dimensional continuum	39, 44	34, 41, 51, 52, 53, 54, 59, 60, 64, 65	43, 66, 67, 69, 75, 77
Two-dimensional continuum	45, 46	40	71
Two-dimensional/three-dimensional discrete	47, 48	25, 49, 55, 56	
Two-dimensional hybrid discrete/continuum		57	68, 70, 76

Re-epithelialization Can Be Manipulated by Altering TGF- β 1 Kinetics

During wound healing, keratinocytes, initially located at the edge of the lesion, crawl over the lesion to restore the protective barrier. Although cell–cell cross-talk is key to this process, external signaling strongly influences the behavior of the leading edge. It overwrites the default program to initiate horizontal growth of skin, temporally bypassing the upward motion of keratinocytes from the basal membrane to the stratum corneum. In this early response of keratinocytes, TGF- β 1 has been recognized as a crucial signaling pathway controlling two major events: it increases keratinocyte migration and decreases keratinocyte proliferation. A balanced action of TGF- β 1 is therefore critical to avoid pathological re-epithelialization. With the help of agent-based computational models, Sun *et al.* (49) explored the role of TGF- β 1 in epidermal wound healing and identified spatiotemporal sequences of events in normal and pathological wound healing. In their baseline model, increased concentration of TGF- β 1 at the edge of the wound induced a population of keratinocytes to migrate inward and blocked their proliferation. As the leading edge moves into the lesion, the adjacent population of keratinocytes was exposed to normal levels of TGF- β 1 but to an increased presence of other growth factors that stimulate cell proliferation. Normal re-epithelialization can thus be viewed as a nonproliferative, highly motile leading edge followed by a proliferative population of keratinocytes. In these computational experiments, disrupting the balance of TGF- β 1 created either chronic wounds or hypertrophic scars (49).

Angiogenesis Creates Fractal Patterns of Vascular Networks

The aspect of wound healing that has received the most attention in modeling is angiogenesis. The reason for the disproportional interest in new vessel formation is that it is not only crucial to wound healing but also to tumor growth. The basic assumptions of angiogenesis models are valid for both circumstances,

and combined efforts between these two scientific communities have markedly pushed the frontiers in mathematical modeling of angiogenesis within the past decade (40,50). Researchers in wound healing and tumor growth have collectively used various approaches to study the creation of new vasculature, and some of their most remarkable models are extremely elaborate. They include most features of the repair sequence: inflammatory cells, one or more chemoattractants, fibroblasts, and the ECM (41,51). In continuum models, new vasculature is represented as the density of endothelial cells in a point-wise fashion or as a combination of capillary tip and sprout densities (52). A partial differential equation dictates the evolution of the endothelial cell density field, in analogy to Fisher's equation, but with additional coupling terms between the endothelial cells and the other fields in their immediate environment (53,54). In discrete models, fractal-like approaches or cellular automata have been proposed to account for the contact between individual endothelial cells as the capillary tubes branch out (55,56). Hybrid models have also successfully reproduced the angiogenesis mechanism (57). The greatest value of angiogenesis models, continuous or discrete, is that they allow us to explore the effects of altered nutrient and oxygen supplies on the healing process.

Angiogenesis Can Be Manipulated by Altering Hyperbaric Oxygen Levels

During wound healing, elevated metabolic needs in the proliferative phase increase oxygen demand. In fact, the lack of oxygen or hypoxia is prevalent in the wound during the inflammatory phase and is an important cue for macrophages to release cytokines that recruit other cell types for the subsequent phases (58–60). If hypoxia is severe, for example in chronic wounds, the tissue is incapable of creating new vasculature, and the healing process is significantly impaired (61). Thus, oxygen therapy has been proposed to accelerate the healing process (62). However, excess of oxygen can also be harmful, because it can lead to

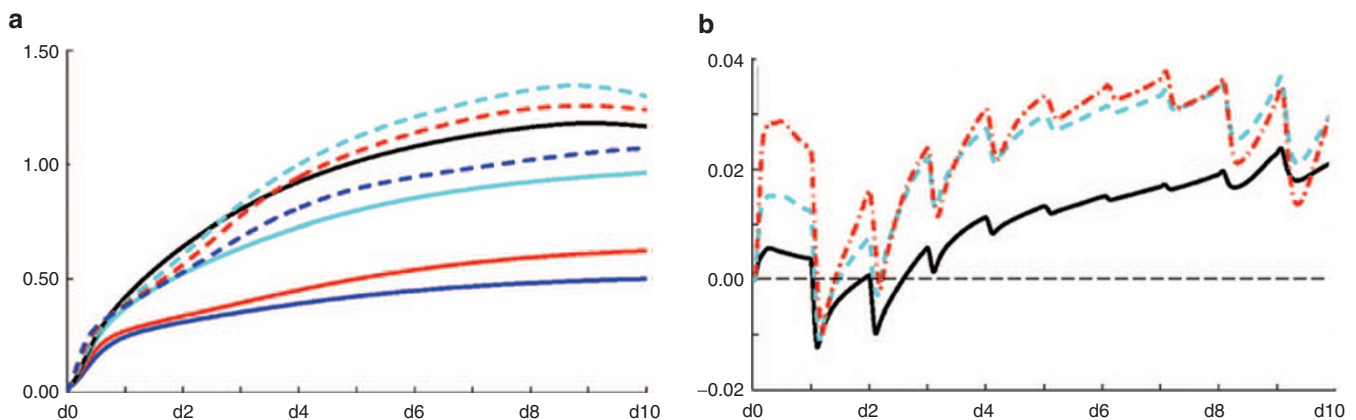


Figure 2. Angiogenesis can be manipulated by altering hyperbaric oxygen levels. (a) The baseline system (solid black line) shows endothelial cell density for oxygen levels in the absence of hyperbaric oxygen therapy during a period of 10 d. Hyperoxic conditions can enhance healing; however, excess of oxygen can be harmful (dashed cyan line: $O_2 = 2.00\%$; dashed red line: $O_2 = 3.00\%$; and dashed blue line: $O_2 = 4.00\%$). Hypoxic conditions impair the healing response (solid cyan line: $O_2 = 0.75\%$; solid red line: $O_2 = 0.50\%$; and solid blue line: $O_2 = 0.25\%$). (b) Hyperbaric oxygen therapy is not constant over the healing period; rather, oxygen is administered daily in short sessions. A common therapy consists of $O_2 = 2.00\%$, 90 min/d (solid black line) leading to increased endothelial cell density over the same 10-d period. Computational simulations can predict alternative therapies (dashed cyan line: $O_2 = 3.00\%$, 90 min/d; and dashed red line: $O_2 = 4.00\%$, 90 min/d). Adapted with permission from ref. 65. Copyright (2008) National Academy of Sciences, USA.

intoxication (63). Hypoxia is an essential cue during the inflammatory phase and is linked indirectly to fibroblast recruitment (62). The interplay of oxygen and cellular recruitment has successfully been studied with the aid of computational models (64).

For example, Schugart *et al.* proposed a model of wound healing in which oxygen tension across the wound is considered as an additional variable (65). Their baseline model reproduced a normal healing response throughout a period of 10 d. Then, different degrees of hypoxia and hyperoxia were simulated. Their simulations revealed that severe hypoxia cannot sustain angiogenesis and that extreme hyperoxia reduces the proliferation of endothelial cells (see Figure 2, top). Finally, they studied different hyperbaric oxygen therapies and concluded that 90 min of hyperbaric oxygen per day enhances the healing process (see Figure 2, bottom).

Wound Contraction by Myofibroblasts Creates Tension in the ECM
 Similar to angiogenesis, collagen deposition and wound contraction have mainly been studied holistically (66,67). Unique

features of their mathematical models are the consideration of fibroblasts and myofibroblasts (68,69). Fully discrete models are not appealing to represent the ECM, and therefore modelers have turned to either hybrid or fully continuous frameworks. In the former, the chemical species and the ECM are modeled as continuous fields, whereas the cells are modeled discretely as individual entities (70). The major focus of hybrid discrete/continuum models has been on collagen deposition. If we decide to also represent cell populations as continuous fields, we can select plain continuum models and more advanced mechanical theories to answer questions such as those regarding myofibroblasts-based active wound contraction (71).

Collagen Deposition Can Be Manipulated by Altering TGF-β Kinetics

Although the biochemical aspects of wound healing have received significant attention, mathematical modeling of the mechanical aspects of wound healing remains largely unexplored. The role of mechanical cues is currently gaining

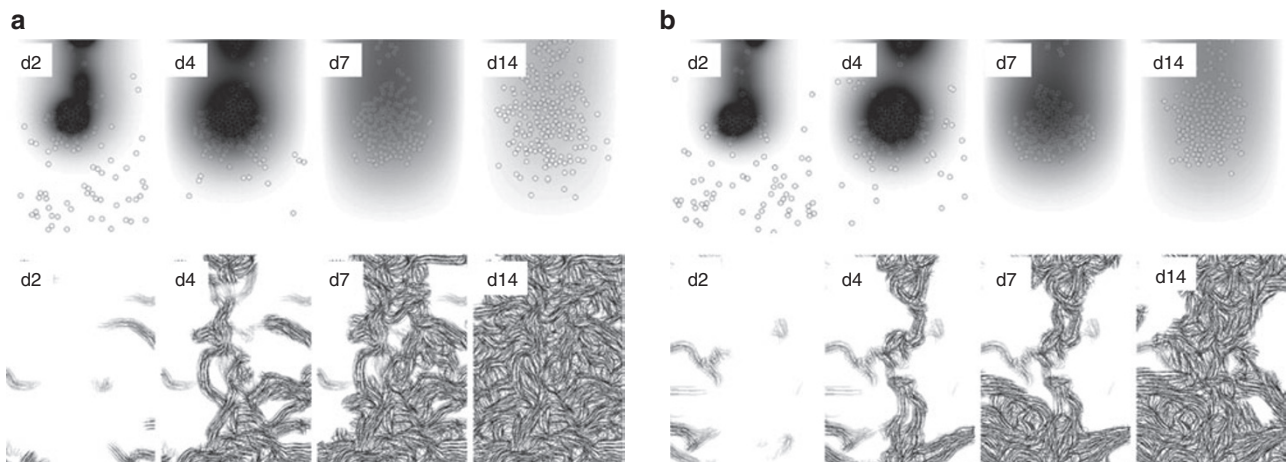


Figure 3. Collagen deposition can be manipulated by altering transforming growth factor-β (TGF-β) kinetics. Two-dimensional hybrid discrete/continuum simulation of wound healing with TGF-β concentration color-coded in gray, top; fibroblasts displayed as discrete cells, top; and collagen fibers displayed through local fiber orientation maps, bottom. (a) Normal TGF-β diffusion initiates progressive collagen deposition at days 2, 4, 7, and 14, whereas (b) reduced TGF-β diffusion generates localized fibroblast concentrations and reduced collagen deposition. Adapted with permission from Figures 7 and 9 in ref. 76.

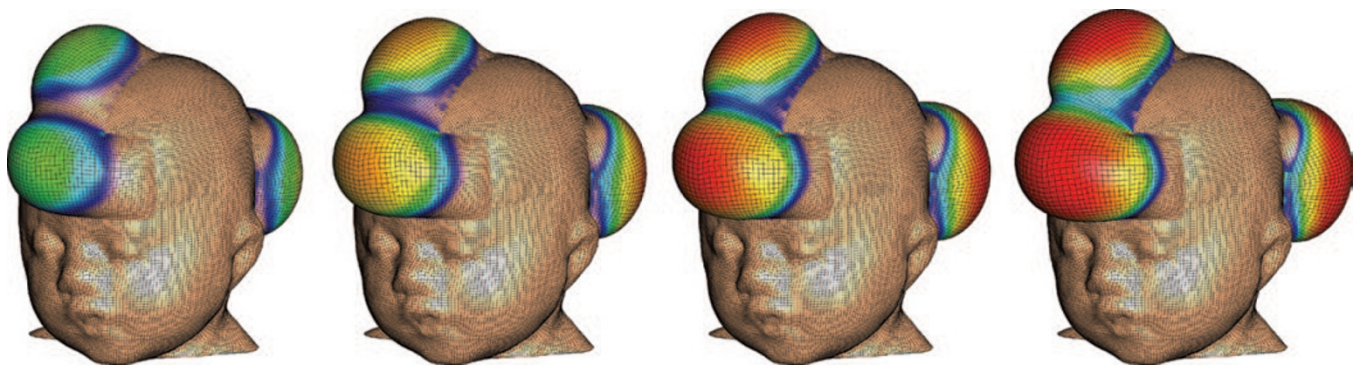


Figure 4. Collagen deposition and mitosis can be manipulated by altering stretch levels. Tissue expansion uses the concept of systematic overstretch to create extra skin for defect repair in reconstructive surgery. Gradually increasing the mechanical stretch over a period of 12 wk, from left to right, triggers a gradual increase in skin area, from blue to red. In the red regions, skin has more than doubled its initial size. From ref. 84.

importance, however, because recent experimental data suggest that releasing mechanical stresses in the wound may shorten the inflammatory phase and reduce scarring (8,72). Mechanical stress is transmitted across the ECM and directly affects fibroblast behavior (73,74). For example, the local environment of a fibroblast can induce its transformation into a myofibroblast (75). Myofibroblasts are responsible for contracting the tissue and bringing the edges of the wound together (69). Although there are a few models that have incorporated wound contraction by myofibroblasts, these models have not yet been used to optimize the mechanical environment and enhance therapeutic outcomes. Nonetheless, because pathologic scarring is a major concern in wound healing, researchers have focused on modeling the flow of chemical information, which regulates collagen deposition and creation of the new ECM (75).

For example, Cumming *et al.* studied the response of fibroblasts to TGF- β , a cytokine released by macrophages during inflammation, throughout a period of 14 d (76). Using a predictive mathematical model, they were able to show that altered TGF- β kinetics in the wound have a significant impact on the healing process. They found that in their baseline model, fibroblasts tend to cluster around the zones with the highest concentration of chemoattractants, where they gradually replace the fibrin clot with collagen as they reach the damaged region (see [Figure 3](#), left). According to the simulations, reduced TGF- β diffusion causes a clustering of fibroblasts, reduced collagen synthesis, and significantly altered healing kinetics (see [Figure 3](#), right). Another remarkable example of the use of agent-based models is the study conducted by Mi *et al.* (77), which focused on TGF- β 1, a specific isoform of TGF- β . Altered kinetics of this chemokine are an important factor in diabetic ulcer pathology. According to their simulations, increased tumor necrosis factor- α and decreased TGF- β 1 lead to an impaired healing response of diabetic patients. The model further showed that altering these chemoattractants increased the concentration of TGF- β 1, increased collagen deposition, reduced concentration of tumor necrosis factor- α , and reduced necrosis.

Collagen Deposition and Mitosis Can Be Manipulated by Altering Stretch Levels

Despite intense research over the past two decades, most existing models for wound healing are still one or two dimensional and focus on the acute rather than on the chronic response (see [Table 1](#)). The majority of these models use finite difference or finite volume methods to discretize their governing equations in space, limiting the geometries to idealized settings. An elegant way to incorporate realistic three-dimensional geometries of the wound and skin anatomy is finite element modeling. Recent trends in computational biology focus on predicting chronic soft-tissue adaptation using mechanistic finite element models (78,79). In an attempt to quantify how elevated mechanical stretch can alter collagen deposition and fibroblast mitosis, several groups have recently modeled chronic skin growth in response to changes in the mechanical environment (80,81). These models have immediate clinical

applications in skin expansion in plastic and reconstructive surgery. Predicting stress, strain, and skin area gain, skin growth models have the potential to enhance treatment for patients with birth defects, burn injuries, or breast tumor removal (82).

For example, Zöllner *et al.* simulated skin expansion in pediatric forehead reconstruction of a 1-y-old girl throughout a period of 12 wk (83). Their model incorporated a thermomechanically consistent representation of the dermis and a phenomenological scalar field that quantified the amount of newly grown skin (82). A conceptually simple and elegant abstraction of the mechanotransduction pathways and the corresponding cellular response defined the evolution of this scalar field in terms of mechanical stimuli such as tissue stretch (83,84) (see [Figure 4](#)). Refining this framework to incorporate the true mechanotransduction pathways during mechanical overstretch or during wound healing appears to be a next logical step toward predicting and improving of wound healing therapies in realistic three-dimensional geometries of pediatric patients.

PERSPECTIVE

Systems-based mathematical modeling of wound healing has achieved remarkable sophistication and is on its way to becoming an irreplaceable tool in personalized medicine. The first mathematical models of wound healing were proposed two decades ago and considered only specific aspects of the healing process. Current models include most of the key components that interact throughout the repair sequence. They have opened the floor to advanced hypothesis testing and enhanced wound management therapies. The state of the art in computational modeling of wound healing is the temporal and spatial integration of different cell types, chemoattractants, nutrients, and the ECM, interacting jointly to restore tissue integrity. Current trends in computational modeling indicate that this knowledge gained on the cellular level should be integrated in holistic multiscale multifield continuum models through a bottom-up approach. The ultimate goal would be to create high-resolution system-level models with parameters calibrated at their generative level of resolution. Rather than fitting physiologically meaningless phenomenological parameters to system-level measurements, which has been the standard for the past decades, all parameters would then have a clear physiological interpretation.

Greatest attention has been paid to the biochemical features of healing such as diffusion of chemoattractants and oxygen tension. The first models have now advanced far enough to reliably predict how systematic manipulations of baseline parameters in chemical signaling can change the healing process. The biomechanical features of healing are currently receiving increasing attention. Mechanical models focus primarily on collagen deposition and active wound contraction. Yet, to date, it still remains unclear how exactly these mechanisms are regulated when the injured skin tries to recover its homeostatic equilibrium state.

One of the major challenges in the mathematical and computational modeling of wound healing is the consideration of complex, physiological geometries in two and three dimensions.

Current models have been tested primarily in axisymmetric conditions, which limits their application to a clinical setting and reduces their translational potential. Another current road block is the incorporation of a detailed mechanical representation of skin with temporally, spatially, and directionally varying material properties. Fortunately, recent advances in molecular biology, mechanotransduction, soft-tissue mechanics, and computational modeling of soft tissues might soon allow us to bridge these gaps. The first patient-specific computational model of pediatric wound healing is likely to appear within this decade, and it will constitute a major breakthrough in the progress of systems biology toward a better care for the pediatric patient.

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