Challenges in the modeling of wound healing mechanisms in soft biological tissues

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Abstract

Numerical models have become one of the most powerful tools in biomechanics and mechanobiology allowing highly detailed simulations. One of the fields in which they have broadly evolved during the last years is in soft tissue modeling. Particularly, wound healing in the skin is one of the processes that have been approached by computational models due to the difficulty of performing experimental investigations. During the last decades wound healing simulations have evolved from numerical models considering only a few number of variables and simple geometries to more complex approximations that take into account a higher number of factors and reproduce more realistic geometries. Moreover, thanks to the improvements on experimental observations, a higher number of the processes that take place during wound healing have been identified and modeled. This work presents a review of the most relevant wound healing approximations, together with an identification of the most relevant criteria that can be used to classify them. In addition and looking to the actual state of the art in the field some future directions, challenges and improvements are analyzed for future developments.

Keywords: wound healing, FE simulation, contraction, tissue regeneration, computational mechanobiology

1 Introduction

Wounds in the skin are a major health matter that most people suffer from during their lives. When the injured person has no serious health problems, wounds heal without any other consequence than leaving a scar in the skin. Nevertheless, when the healing process does not follow its natural evolution, several complications arise, leading to chronic wounds and other healing disorders. Millions of surgical procedures are performed every year with the subsequent wound and scar treatment. Chronic wounds
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affect around 6.5 million patients in the United States causing an annual cost of US $25 billion. It is also calculated that skin scarring care generates an annual cost of around US $12 billion. The reduction of these costs is an ongoing issue within the health system in the US for the health system.

Wounds in the skin can appear as a consequence of damage in traumatic accidents but also as a result of surgery incisions which are sometimes difficult to heal. Moreover, wounds such as pressure ulcers may appear caused by long periods of immobility and which may lead into infections and more severe or even fatal complications. Complicated wounds are more likely to affect people with chronic diseases such as diabetes and obesity, factors which present added difficulties for a proper healing.

The search for improved healing methods is time consuming and results in high economic cost. Moreover, ethical issues always arise when experiments involve living samples. As a consequence, mathematical models of wound healing have become more popular during the last decades. Mathematical models allow the studying of a wide number of cases by a parameter sensitivity analysis within a short period of time and with low economic cost, adding the possibility of studying specific cases for individual patients. Therefore, many researchers aim at the development of wound healing models that predict the healing evolution of wounds under different conditions. However, the existing models still present limitations that reduce their potential, and they should be thoroughly analyzed to understand the main challenges and difficulties that must be undertaken in order to improve their predictive capacity.

Nevertheless, we have to keep in mind that different numerical strategies have been employed to simulate wound healing phenomena, from continuum to discrete approaches. In this work, we mainly focus our analysis on the main difficulties found on continuum approaches. However, some authors have simulated wound healing closure using particle-based models where they simulate each cell as
a particle, incorporating different interactions, mainly, cell-cell and cell-extracellular matrix (ECM) interactions. In all these cases, authors consider the matrix as a continuum solid. However, it is also interesting to remark that there are other authors that have considered a discrete approach for both the cell population and the ECM.

Thus, the aim of this paper is to review continuum models and classifying them by attending to different aspects (geometry, material and regulatory stimuli) in order to identify possible improvements. First, an introduction on wound healing will be given in order to understand the process and to present the variables that guide this regenerative phenomenon. Thereafter, a review of the existing models together with their classification according to the previously mentioned aspects will be presented. Finally, an analysis of the possible improvements for future models will be discussed.

2 Biology of wound healing

Wound healing takes place after damage occurs in the skin. Epidermal wound closure is due only to the migration of epidermal cells migration and it is not affected by wound contraction which only takes place when deeper layers of the skin are affected. Minor wounds that reach the deeper layers heal usually through a series of well organized processes without special treatment, but in those cases in which wounds cause an excessive damage, it is necessary to apply different techniques to achieve healing. Besides, abnormal healing processes, such as fibroproliferative diseases (keloids or hypertrophic scars) or chronic wounds (venous ulcers, pressure ulcers, and diabetic foot ulcers), can not lead to a proper healing without external help due to their aberrant physiological activity.

Wound healing is usually divided into three stages which are overlapped in time and which can last for
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several months or years. These stages are: inflammation, tissue formation and tissue remodeling. Inflammation begins when the injury appears (Figure 1 A). After a wound occurs, extracellular matrix (ECM) is replaced by a blood clot made of blood from disrupted vessels. Inflammatory factors such as platelet derived growth factor (PDGF) are secreted by platelets to stimulate cellular activity. The vessels are subsequently closed and bleeding stops (hemostasis). A hypoxic fibrin clot then replaces the actual blood clot. Inflammatory cells (neutrophils and macrophages) clean the wound site and eliminate bacteria, dead tissue and other foreign particles. Inflammation lasts for approximately 48 hours and growth factors such as TGF-β and VEGF, necessary to promote cell activity in the next stage, are released thereafter.

Epithelialization, or new tissue formation, begins some hours after the wound occurs and lasts between 2 to 10 days (Figure 1 B). This stage is characterized by the migration and proliferation of several cellular species, mainly fibroblasts and endothelial cells, into the wound site, driven by the growth factors released at the end of the inflammatory stage. The temporary clot is removed and replaced by a granulation tissue. At a later stage, the granulation tissue is substituted by a new extracellular matrix mainly made of collagen. Fibroblasts that have infiltrated the wound site are activated and differentiate into myofibroblasts. Both cellular species initially secrete collagen that forms a disorganized fiber network, which gives mechanical support to the new tissue. At this stage, epithelial cells stimulated by VEGF form new blood vessels from the damaged ones, a process known as angiogenesis. This process allows the re-establishment of the normal blood and nutrients flux to the tissue and also provides the oxygen supply necessary for cellular activity.

Moreover, the new extracellular matrix that contains vessels and several cell types is contracted during this stage. The contraction of the tissue is due to the forces that cells (fibroblasts and myofibroblasts)
exert in response to the change in the material properties in the damaged area. Myofibroblasts are non-motile cells which are able of exerting higher traction forces than fibroblasts. Thus, an adequate proportion between both cell types is crucial for avoiding healing disorders\(^\text{59}\). Once reepithelialization is completed, superfluous cells disappear from the granulation tissue, mainly by apoptosis. Once this process is concluded, an initial scar is formed.

Remodeling begins once the collagen density within the wound reaches that of the undamaged skin (Figure 1 C). At the beginning of the process, collagen fibers are randomly oriented. However, as time evolves, collagen fibers tend to orientate along preferred directions, usually parallel to the skin tension lines. Tissue properties evolve, thereby increasing the damaged skin’s tensile strength towards that of healthy skin. Although tissue functionality is mostly recovered after several months to years, a complete recovery is never achieved because the properties of the newly formed tissue remain weaker than the properties of the healthy tissue.

3 Mechanosensing and mechanotransduction in wound healing

It is well known that physiological processes depend on multiple biological and chemical factors. For example, the force generated by myofibroblasts depends on the transforming growth factor $\beta_1$ (TGF$-\beta_1$) dose\(^\text{65}\), as it regulates the expression of $\alpha$-SM actin. Moreover, TGF$-\beta_1$ also stimulates fibroblast differentiation into myofibroblasts and increases collagen production\(^\text{47}\).

These processes are influenced by mechanics as well. Experimental evidence\(^\text{28,38}\) suggests that cells feel and react to mechanical stimuli from their environment (mechanosensing) in order to regulate their activity and to turn their environment into a more comfortable one. Mechanical forces such
as stretching tension, shear force, scratch and compression, among others, are perceived by cellular mechanoreceptors and mechanosensors. Similarly, cells can translate biochemical information into mechanical activity (mechanotransduction). It has been proved that processes like cell migration, differentiation or force generation are influenced by mechanical stimuli.

The role of mechanical tension in fibroblast differentiation was shown in full-thickness wounds in rats. In their work, Hinz et al. found that higher tension induced greater tissue contractility and myofibroblast markers, which should be a consequence of the formation of stress fibers. Furthermore, α-SMA expression is also favored by mechanical tension.

4 Modeling of wound healing

A number of aspects must be carefully taken into consideration when proposing continuum mathematical models for the simulation of the healing process. These model characteristics will not only define the accuracy of the results but also the computational requirements, and will to a certain extent limit the predictive capacity of the model.

Mathematical models of biological processes usually come from the same group of seminal models. Although authors extend these models by adding new factors and variables, sometimes the limitations of the original model are transmitted to subsequent models.

Most of the existing wound healing models are described by a set of convection-reaction-diffusion equations that define all the processes involved during healing. The first wound healing models only included the influence of biological factors (cellular species and chemical substances, Figure 2). The evolution of the cellular and chemical species is obtained from a balance equation defined by a general
relation

\[ \frac{\partial Q_i}{\partial t} + \nabla \cdot J_{Q_i} = f_{Q_i}(Q_1, Q_2, \ldots, Q_n) \]  

(1)

where \( Q_i \) denotes the \( i^{th} \) cellular/chemical species. The vector \( J_{Q_i} \) denotes its net flux over the domain of interest including terms due to random dispersal (migration or diffusion) and directed migration (chemotaxis) and \( f_{Q_i} \) denotes the species net production, accounting for cellular proliferation, differentiation, dedifferentiation and death, and production and degradation of the chemical substances.

Note, that the model may consider generally an arbitrary number of species, and each of them may influence the flux and production of the others.

Sherratt and Murray\(^{56}\) proposed the first reaction-diffusion model for epidermal wound healing by setting the interaction between a mitotic growth factor (activator or inhibitor) and the colony of epidermal cells that produces it. Furthermore, cell variation arises from migration and mitosis regulated by the produced chemical factor and also by apoptosis\(^{6,16}\). Moreover, Sherratt and Murray\(^{56}\) proposed the first model that included chemical control of cell function, and it has been the base for most of the subsequent wound healing and contraction models. Moreover, their model has also been adapted to simulate wound healing in other tissues. For instance, Dale et al.\(^{7}\) adapted this model in order to study corneal epithelial wound healing as a function of the epithelial growth factor (EGF).

In addition, epidermal wound healing has been simulated together with other processes that taking simultaneously, such as angiogenesis\(^{35}\).

For as long as healing models have evolved, works have included the effect of mechanics, coupling the biological and the mechanical behavior of the skin. Wound contraction is one of the most important
processes during dermal wound healing and it is highly influenced by mechanics. When the influence of mechanics is included in the model, the conservation law (Eq. 1) for each species incorporates a passive convection term in the net flux, $J_{Q_i}$, due to the extracellular matrix deformation.

The matrix deformation is obtained from the balance of linear momentum, where the passive resistant ECM stress (defined according to the mechanical material model adopted to characterize the mechanical behavior of the skin) and the ECM stress due to the cells-ECM adhesions equilibrate the ECM-substrate anchoring forces resisting ECM deformation. Including mechanics allows the quantification of the reduction of the wound size due to the inward retraction of the wound contour.

The first wound contraction model was proposed by Tranquillo and Murray and it has been so far the base of most wound contraction models. The model was composed by a set of differential equations describing the evolution of a cellular species (fibroblasts), conservation of the ECM collagen density and the linear momentum of the matrix. Tranquillo and Murray included the effect of cell growth, migration and diffusion together with the passive convection due to the ECM movement. During a first approach they considered that the ECM variation was due only to its passive convection. At a later stage, Tranquillo and Murray introduced an ECM evolution law which included ECM synthesis by fibroblasts. Moreover, in the same work, they proposed a more complete biochemical model including the chemotactic effect of a chemical growth factor.

The model by Tranquillo and Murray was extended to include the effect of myofibroblasts on the contraction of the wound. It has been reported that a proper contraction level is not reached without myofibroblasts forces. Olsen et al. proposed a wound contraction model that included the displacement of the contracted tissue and applied it to normal and pathological wounds. Olsen et al. described the temporal evolution of fibroblasts, myofibroblasts, a chemical growth factor and
the extracellular matrix during wound contraction. Fibroblasts and myofibroblasts evolution laws incorporated mitosis, differentiation, apoptosis and passive convection. Fibroblast migration also included random dispersal and chemotaxis. They predicted the evolution of the wound all along until it had reached a steady state.

Following the work by Tranquillo and Murray, Murphy et al. proposed a more complex model that, in addition to the TGF-β kinetics, also considered the effect of collagenase (an enzyme that contributes to new tissue formation) on the collagen concentration. One of the main differences from the Murphy et al. to the previous models was the differentiation mechanism from fibroblasts to myofibroblasts. Moreover, Murphy et al. assumed that myofibroblasts generate traction forces even in the absence of fibroblasts, as experimental evidence shows. Unlike previous models, they also assumed that there is no differentiation from myofibroblasts back to fibroblasts.

Murphy et al. developed a model that was able to reproduce the interaction between the cellular, chemical and mechanical phenomena by adding new factors. The critical role of TGF-β on dermal repair was incorporated through the differentiation mechanism from fibroblasts to myofibroblasts, which was activated by both TGF-β and tissue stress. Murphy et al. applied their model to investigate certain healing disorders. Thus, when there is excessive TGF-β a contracture can appear caused by excessive myofibroblasts forces. On the other hand, the wound contracts insufficiently when TGF-β disappears too quickly and when there are not enough myofibroblasts to generate forces. They came across the same effect when the myofibroblast kinetics were modified by changing the myofibroblasts differentiation from fibroblasts and the myofibroblasts death rate.

Following the work of Olsen et al., Javierre et al. proposed a mathematical model of wound contraction by including fibroblasts, myofibroblasts, collagen, a generic growth factor and the tissue
displacements. They included the effect of mechanical stress to regulate cellular processes, defining
the traction stresses generated by cells through the concept of net stress of one fibroblasts cell per unit
of ECM matrix as introduced by Moreo et al.\textsuperscript{39}. They proposed a mechanosensing model applicable
to cellular processes such as migration or proliferation, based on the Hill's model for skeletal muscle
behavior. In their work, Moreo et al.\textsuperscript{39} proposed a model to evaluate the octahedral stresses exerted
by cells as a function of the tissue stiffness. They evaluated this stress through two components. The
first one measures the contractile stresses generated by the myosin machinery transmitter thorough
actin bundles and the second term is related to the contractile stress supported by the passive re-

tance of the cell (absorbed by the microtubules). These two contributions quantify to the stress
that the cell effectively transmits to the ECM. This approach considers that the strain to which the
substrate and the cell are subjected to is the same. Javierre et al.\textsuperscript{30} also included this factor in the
expression of fibroblast differentiation into myofibroblasts, as it had been experimentally observed
that differentiation is guided by mechanical tension\textsuperscript{27,59}.

Valero et al.\textsuperscript{62} adapted the model by Javierre et al.\textsuperscript{30} in order to simulate deep wounds. Valero
et al.\textsuperscript{62} used the same biochemical approach but modified the mechanical conditions to adapt the
model to the new geometry. In a successive work, Valero et al.\textsuperscript{63} proposed a new formulation of
fibroblast to myofibroblast differentiation and fibroblast (and myofibroblast) traction forces based on
mechanosensing and mechanotransduction principles.

The presented models can be classified according to their attention to different aspects, particularly
with respect to wound geometry and material model (Figure 3). Investigating different numerical
models that include each characteristic and their potential, it is possible to find the directions in
which future models could be oriented.
4.1 Geometrical approaches

According to the proposed categories (see Fig. 3), the first aspect that can be used to classify the existing wound healing models is the geometry (i.e. morphology) of the wound to be simulated. Most wound healing models are limited to one-dimensional (1D) geometries. This oversimplification of the spatial domain is useful to study simple axisymmetric geometries (such as straight or circular superficial wounds) with less computational cost. In fact, one-dimensional models offer the possibility of providing a first examination of the interaction between biochemical factors at the cellular level or therapies, although their true predictive power is limited. For instance, the wound morphology remains axisymmetric over time, which differs from reality given the anisotropic nature of the skin.

The seminal one-dimensional models of epidermal wound healing and wound contraction have recently been extended to two dimensions. In the case of wound contraction, this extension implies the formulation of the proper boundary conditions and the use of advanced numerical techniques to solve the nonlinearly coupled equations. Two-dimensional (2D) models allow the consideration of wound morphologies that remain uniform in the third spatial dimension. Hence, with two-dimensional models one can simulate arbitrary superficial wounds or elongated surgical wounds. The hypotheses of plane stress or plane strains shall be adopted to model the mechanical evolution of the ECM in superficial and deep wounds, respectively. On the other hand, modeling deep wounds implies dealing with a free boundary given by the external surface of the wound and the undamaged tissue.

The above-mentioned two-dimensional models offer a closer approximation to the wound morphology...
and allow the analysis, to some extent, of the effect of wound shape in the healing pattern. However, three-dimensional models are required to obtain an accurate representation of the involved phenomena. Unfortunately, their proximity to real medical application comes along with an elevated computational cost. Despite their great potential, and as far as we know, there is only a single three-dimensional model of wound healing \(^6\) which focused on the contraction of an ellipsoidal wound.

### 4.2 Material constitutive laws

The mechanical properties of the skin have been studied for centuries. One of the first experiments in this field was performed by Langer \(^3\), illustrating that skin is naturally subjected to anisotropic stresses. Skin pre-stress is clearly observed when a wound occurs and the skin relaxes and loosens, causing an increase in the initial defect size.

Moreover, the mechanical properties of the skin also vary depending on the skin location, orientation and depth, but also on age. Skin loses its elasticity and recovery capacity along time \(^1\). Most of the mechanical properties of the skin are due to the fibers that compose its ECM, which have an elastic modulus of 150-300 kPa \(^7\). Matrix fibers have a high tensile strength derived from the organization of three primary protein chains (collagen, elastin and fibrin) into a superhelix \(^3\). The main components of these fibers are collagen, which gives most of the tensile strength to the ECM and elastin which gives elastic properties to the skin and allows it to recover to its original state after being stretched.

Protein fibers are embedded in a ground substance made of proteoglicans and fibronectins \(^1\), that helps cells to move through the fibers. Collagen fibers are aligned in the skin following the stress lines or Langer lines \(^3\). Langer \(^3\) performed circular cuts in the skin on the entire body surface, finding that these cuts turned into ellipses aligned with tension lines when the skin relaxed. The orientation
of these cuts defined the natural orientation of collagen fibers, usually parallel to the underlying muscles. Wounds parallel to Langer lines heal better and produce less scarring while wounds that are perpendicular to Langer lines show more profound healing difficulties. Moreover, the magnitude of the skin pre-stress found in Langer experiments has been measured in the arm and forearm by Flynn et al., where they found values ranging from 28 to 92 kPa. Further knowledge of the skin properties has been an important issue for experimental researches, and several measurement methods have been reported. During the last decades, both in-vivo and in-vitro experiments have been designed to characterize the mechanical behavior of the skin and to find accurate parameter values. Most of the in-vivo studies to measure skin properties are carried out in the forearm or the upper arm and use different mechanical assays such as extension, indentation, suction or torsion. Diridollou et al. used suction tests in the forearm and an inverse method to identify the nonlinear material parameters of the skin. They applied negative pressure to the surface of the skin and measured the skin deflection, finding that the skin becomes stiffer when it suffers higher strains. Boyer et al. studied the mechanical properties of the epidermis and the dermis by using a micro indentation device and characterized it as a viscoelastic material, finding that the complex modulus has values of 47.3 to 128.3 N/m. Silver et al. studied the viscoelastic properties of the skin components from experimental data. They estimated that the elastic constant for skin collagen is 4.4 GPa and for the elastin it is 4.0 MPa on thoracic and abdominal skin. Other works characterize skin as a hyperelastic material by considering anisotropy and incompressibility. Flynn et al. measured the force-displacement response in the forearm skin when three-
dimensional deformations were applied. They later found values for the material parameters that
fit the Ogden hyperelastic model and the Tong and Fung model\textsuperscript{15}. Gahagnon et al.\textsuperscript{17} studied the
anisotropy of forearm skin in-vivo by using elastographic tests. They stretched the skin parallel and
perpendicularly to Langer’s lines and found anisotropic behavior.

While in-vivo studies provide information on the skin in its natural environment, in-vitro studies
provide more controlled experiments, where different aspects can be isolated. Annaidh et al.\textsuperscript{2}
investigated the influence of location and orientation of the skin, focusing on skin anisotropy. They
performed in-vitro tensile tests on human skin samples obtained from different parts of the back, finding a correlation between the orientation of Langer lines and collagen fibers. Later, Annaidh et al.\textsuperscript{1}
matched their results with the anisotropic hyperelastic model developed by Gasser et al.\textsuperscript{18}.

Groves et al.\textsuperscript{21} used a tensile test on circular human skin specimens to perform tests along three
different load axes. They found that skin shows anisotropic hyperelastic behavior and used the model
by Weiss et al.\textsuperscript{70} to characterize it.

Moreover, computational models used to simulate skin behavior is without considering any kind of
disorder usually adopt a hyperelastic approach\textsuperscript{5,34,26,1}. Nevertheless, to the knowledge of the authors,
there is no experimental work that analyzes the mechanical properties of the wounded skin.

First epidermal\textsuperscript{56} and dermal\textsuperscript{60} healing models focused only on the influence of biological processes
and factors. To also take into account the influence of mechanics on these models, they subsequently
included the mechanical characterization of the skin\textsuperscript{45,30}. A viscoelastic material model has traditionally been adopted for modeling the mechanical behavior of the skin in wound healing\textsuperscript{45,36,30,72,42}. This approach captures some time-dependent properties of the skin but also presents some limitations.

Mechanical properties of the skin are usually updated with an evolutive law. For instance, the elastic
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Modulus has been taken proportional to the collagen density in several works\textsuperscript{30,42,63}. However, implementing skin as a hyperelastic material would allow the reproduction of the healthy skin behavior, including the possibility of adding tissue anisotropy. There are a few wound healing models that use hyperelasticity to reproduce the skin behavior, by considering isotropic properties\textsuperscript{61} or anisotropic properties\textsuperscript{64}. Experiments have proved that a hyperelastic constitutive material model reproduces properly the skin behavior\textsuperscript{2}, although its characterization complicated. To observe the influence of anisotropy on the process, it should be combined with three-dimensional geometries. The influence of the anisotropy on certain directions would otherwise be lost.

4.3 Regulatory stimuli modulating cell behavior

The mechanical stimulus which guides the evolution of cell differentiation, contraction and matrix synthesis is another aspect that can be used to classify wound healing models. Most existing models use phenomenological laws to regulate these processes. Murphy et al.\textsuperscript{42} established that the mechanical stimulus which guides differentiation was the positive elastic stress. This idea was first introduced by Hall\textsuperscript{24}, while previous approaches proposed that this stimulus was related to the cell traction stress\textsuperscript{30}. They followed the approach proposed by Tranquillo and Murray\textsuperscript{60} i.e., to evaluate the traction forces exerted by cells by considering them proportional to the collagen density and the cell concentration and by neglecting the saturation terms dependent on cell densities\textsuperscript{60} or ECM densities\textsuperscript{45,30}. Even though these models are able to capture the global evolution of wound contraction, the proposed laws produce high traction forces where there is low concentration of cells or ECM. Valero et al.\textsuperscript{63} proposed a physically evidenced law for fibroblast differentiation into myofibroblasts, that depends on the volumetric strain ($\theta_{cell}$) that fibroblasts are
supporting. They also assume fibroblasts differentiate only when they are stretched.

More recently, Brugues et al. \textsuperscript{4} constructed an in-vitro and computational model in order to understand how the cell forces during migration regulate wound contraction, concluding that ring tension has to be not uniform in space in order to predict higher tangential forces at the wound edge as they have experimentally quantified.

5 Future directions

Understanding the wound healing process in order to reproduce it by computational models is crucial for advancing in the development of healing solutions and surgical procedures. Moreover, when modeling the healing process, decisions concerning the model characteristics must be taken in order to obtain the most suitable results.

Thus, it is of great importance to understand the healing mechanisms in order to propose novel treatments that favor the healing process and prevent complications, such as ulcers or fibroproliferative disorders. These advances include the application of different growth factors or drugs to patients, the choice of better incision geometry in surgery that helps healing, creating less scaring, or the development of suture techniques that allow the minimization of scar formation \textsuperscript{25,48}. Nevertheless, when normal healing does not even occur with aid, more complex and expensive techniques must be applied.

There are several aspects which should be improved so as to predict healing according to the present necessities considering the capacity of the existing healing models.

The possibility of simulating wound geometries without simplifications is crucial. It is well known that stresses and deformations created in the skin during healing and, subsequently, scar size and
shape depend on the initial wound morphology and location. Every anatomical location has specific
properties such as skin thickness, composition, elasticity and residual stresses. Moreover, the different
mechanical properties of the skin layers should be considered in the model together with their thick-
nesses. In addition, the different muscles or tissues under the skin affect the wound evolution and thus,
they should be included in simulations to consider their effect. In this direction, a first step has been
done by including the healthy skin fibers in the simulations\textsuperscript{64}. The relative orientation of the wound
in relation to these fibers define the directions in which the wound is loaded. Moreover, the final scar
that the wound is going to leave depends on the last healing stage, remodeling, which has not yet
been included in any healing model. The simulation of the complete healing process is necessary to
provide a useful tool to clinicians, as the final scar appearance is not reached until months after the
wound is produced, in the remodeling stage.

Another open field in which computational models may provide valuable insights is the study of wound
pathologies, in which healing is impaired, excessive or which presents other difficulties such as pressure
ulcers or keloids. There exists a number of models that study the adaptation of the mechanical
properties of the skin when a disorder appears. For example Guiotto et al.\textsuperscript{22} studied the diabetic
neuropathic foot mechanical properties compared to a healthy foot. The evolution of pressure ulcers
and the prediction of the mechanical environment in which they are more likely to appear has been
also studied\textsuperscript{32}. The study of these cases could be simulated by properly identifying and adjusting
the biochemical kinetics or the skin properties. Recently, Xue et al.\textsuperscript{72} developed a wound healing
model in which they compare the healing of ischemic and non-ischemic wounds. In-silico results were
successfully validated against the experiments of Roy et al.\textsuperscript{52}.

Future directions also account for the simulation of assisted healing. There are wounds that do not
heal without treatment. Thus, the development of models that include healing solutions, such as sutures or other devices that modify the mechanical conditions of the affected tissues, are of great value. Moreover, the application of topical growth factors that stimulate healing can be included in the actual models to predict their effects. The simulation of these phenomena will considerable help clinicians in order to choose favorable solutions in specific cases.

Finally, in-silico models cannot improve their prediction capacity without a proper feedback from experimental works. Both, in-vivo and in-vitro experiments can facilitate the identification and estimation of the most relevant variables of the healing process. In-vitro techniques such as two-dimensional cell cultures can be used for the study of isolated cellular processes and for the quantification of the parameters that define the model. Experimental works help to validate computational models and can make it possible to determine how close the wound evolution predicted by the model is to reality. There are only a few in-vivo experiments that measure the evolution of wounds in animals. Unfortunately, the ethical restrictions together with the high variability of the subjects have prevented these experiments from being continued. However, it should be taken into account that computational models usually focus on wounds in humans and thus the need of an easy extrapolation of the results from experimental works is crucial. Animals such as rats show a faster healing than humans, also mechanical properties are different among species. Thus, it is only possible to catch the general tendency of wound healing in such experiments and parameters should be adjusted to be used in human models.

Thus, despite the fact that computational modeling of wound healing has substantially evolved in the last years, adding great improvements to the seminal works, there is still room for further developments and research.
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Figure 1: Stages of wound healing. A) Inflammation. B) Tissue formation. C) Tissue remodeling.
Figure 2: Cellular and chemical species scheme in the wound and the surrounding healthy skin.
Figure 3: Classification of wound healing models attending to different aspects.