Computational Modelling of Wound Healing Insights to Develop New

M. J. Gómez-Benito, C. Valero, J. M. García-Aznar and E. Javierre

Abstract About 1% of the population will suffer a severe wound during their life. Thus, it is really important to develop new techniques in order to properly treat these injuries due to the high socioeconomically impact they suppose. Skin substitutes and pressure based therapies are currently the most promising techniques to heal these injuries. Nevertheless, we are still far from finding a definitive skin substitute for the treatment of all chronic wounds. As a first step in developing new tissue engineering tools and treatment techniques for wound healing, *in silico* models could help in understanding the mechanisms and factors implicated in wound healing. Here, we review mathematical models of wound healing. These models include different tissue and cell types involved in healing, as well as biochemical and mechanical factors which determine this process. Special attention is paid to the contraction mechanism of cells as an answer to the tissue mechanical state. Other cell processes such as differentiation and proliferation are also included in the models together with extracellular matrix production. The results obtained show the dependency of the success of wound healing on tissue composition and the importance of the different biomechanical and biochemical factors. This could help to individuate the adequate concentration of growth factors to accelerate healing and also the best mechanical properties of the new skin substitute depending on the wound location in the body and

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its size and shape. Thus, the feedback loop of computational models, experimental works and tissue engineering could help to identify the key features in the design of new treatments to heal severe wounds.

Keywords Wound healing · Mechanobiology · Wound contraction · Skin substitutes

1 Introduction

Skin is the largest organ of the human body. It represents the natural interface between the body and the environment, acting not only as a physical barrier but also as a regulator of water loss. It is part of the immune system, avoiding the entrance of strange particles and pathogens. When skin is injured, it heals through a complex cascade of events aimed at restoring skin integrity. Normally, wounds heal without additional complications in the time curse of a few weeks (to recover the main functionalities of skin). However, when the healing path is altered (by many pathological conditions affecting this process), severe or chronic wounds appear. These severe wounds include pathological scars in which there is an overexpression of collagen (hypertropic scars and keloids), contractures in which there is not enough scar formation, large area wounds such as burns, and chronic wounds such as pressure ulcer. The treatment of these severe wounds represent a high socioeconomical cost. For example, it has been reported that in the year 2000, the treatment of pressure ulcers reached the 4% of the United Kingdom National Health System expenditure [2]. In the same country, in the year 2011, the average cost of a pressure ulcer ranged from £1,214 to £14,108, attending to the severity of the case [9]. It is also important to notice that these injuries also cause considerable pain to the patient, can add months to the hospital stays [9], and in the worst scenarios dramatically reduce the quality of life of the patients. Thus, it is really important from a societal, clinical and economic perspectives to put our efforts in the prevention and reduction of the number of severe wounds and improve, in the range of our possibilities, their treatment.

Serious injuries to the skin do not heal autonomously, and usually they require coverage to repair and restore skin function or other external treatments such as negative pressure wound therapy. Skin replacement is normally done by autologous skin graft of a patient; healthy skin is separated from the donor site and transplanted into the recipient area. However, availability of sufficient healthy skin can limit this treatment, as well as the additional health risks associated with it such as the deformation of the donor-site [39]. Allogenetic, xenogenetic, syngeneic and cadaver skin grafts are also used; nevertheless, they present also problems of availability and add the problem of rejection.

Due to the above mentioned limitations of skin grafts, engineering skin substitutes emerge as a growing area in both clinics and engineering research. However, currently, no skin substitute has provided an outcome similar to an autograft [39]. Some of the problems with existing skin substitutes are the reduced vascularization

and scaring of the graft margins. Moreover, their structure remains to be relatively simple (normal single layer or bilayered), and their three dimensional architecture and mechanical properties are still far from those of skin. This is in part due to the processes employed to fabricate them, being technologically extremely difficult to replicate the way healthy skin appears during embryogenesis and fetal development [54]. In order to improve these treatments it is really important to know the mechanical behavior of healthy skin and the wound, and how they influence the cell mechanosensing capacity. Therefore, the next step in tissue engineering shall be the design of new skin substitutes able to replicate skin and wound mechanical and functional characteristics.

Other treatments for chronic wound include the application of external stimulation. For example, negative pressure wound therapy (NPWT) has been used to promote healing of severe injuries, and it is widely extended in chronic wounds [22]. This treatment is based on the positive effect of mechanical stimulation in the healing of tissues. The positive effect of tension strains has been observed to regenerate other biological tissues such as bone in the distraction osteogenesis process in which the rapid vascularization is a key factor in the development of new bone [47]. Oxygen-based therapies have been explored for the treatment of chronic wound in diabetic patients. In this respect Hyperbaric Oxygen Therapies (HBOT) seek the promotion of healing by the stimuli of the angiogenesis process [12], although the inherent negative effects of this therapy need to be further examined.

Unfortunately, we are still far from developing a definite treatment to heal severe wounds. The traditional approach to develop new therapies has been based on the use of animal models. However, animal experimentation is expensive in economical, social and temporal terms. Also, there is no animal model of skin which completely mimics all the characteristic of human skin [31]. On the other hand, the use of *in silico* models offers a wide (and cheap) spectrum for analyses. Consequently, it is called to play a prominent role in the design of new treatments, due to its intrinsic capacity to isolate single factors [18].

2 Healthy Skin

Skin represents a 6% of the total body weight [56], it has a variable thickness depending on the body location from around 1.5 mm in the sclap to 4 mm in the back. It is a highly organized structure consisting of three main layers: the epidermis, the dermis and the hypodermis.

The most external layer of the skin is the **epidermis** which is continually renewing. It is approximately 75–150 μm in thickness. The epidermis is also divided into five layers. The stratum corneum is the outermost layer of the epidermis which provides a permeability barrier and is mainly composed by a mixture of lipids and an acid mantle important to prevent infections. The other four layers which compose the epidermis are the stratum lucidum, the stratum granulosum, the sratum spinosum and the stratum germinativum which are a second-line epidermal barrier between the

outside and inside of the body [1]. The main epidermal cell types are keratinocytes which represent around 90–95% of the present cells. Keratinocytes are antinflamatory mediators which are part of the immune system. Other cells types in the epidermis include melanocytes responsible of skin pigmentation and protecting against damage of UVR radiation, Langerhans cells which maintain immune homeostasis in skin and represent around 5% of the epidermal cells [50] and Merkel cells mechanosensory cells which participate in touch reception [34].

The epidermis and the dermis are tightly connected through the **basement membrane**. This membrane represent a critical interface which allows cell communication and regulates different cellular activities such as differentiation and apoptosis. It has a structural role, maintains the architecture of the tissues and also provides anchorage for cells migration, macromolecules transit and temporal storage of these macromolecules [36]. The basement membrane is mainly composed by collagen type IV, laminin, proteoglycan and tentactin [6].

The **dermis** is the second skin layer, it is a dense fibroelastic connective tissue of around 1–4 mm thickness. It can also be divided into two layers: the outer one is the papillary dermis which contains disorganized collagen fibers; and the inner one is the reticular dermis, which is formed by organized fibers. The papillary dermis is thinner that the reticular one. The dermis contains much less cells than the epidermis and it is in charge of housing skin's appendages (eccrine and apocrine sweet glands, pilo-sebaceous follicles), nerves, vascular and lymphatic vessels and receptors [56]. The predominant cell population of the dermis is the fibroblast which produces and degrades the extracellular matrix and also contracts it. Other cell populations in the dermis are dendritic dermal cells, macrophages and mast cells which are multifunctional cells of the immune system. The dermis gives structural support and mechanical strength to the skin, and it is also responsible of the nutritional supply. The extracellular matrix of the dermis is composed by different fibers: collagen and elastic fibers [58]. Collagen provides tensile properties to the dermis, being collagen type I the most abundant, however there are also other collagen types (III, V). In addition, elastic fibers gives elasticity to the dermis, which include elastin, fibrillin and microfibrilar components. Finally, the extrafibrilar matrix components of the dermis are proteoglycans which maintain water and ion balance, glycoproteins, water and hyaluronic acid [58].

The third layer, the **hypodermis** (or subcutaneous fat) is composed of loose fatty connective tissue. It serves to fasten the skin to the underlying tissue, it represents an energy reserve for the body and it is also a thermoregulator. The main cell population of the hypodermis is adipocyte.

The collagen fibers of skin are not isotropically oriented [26]. In fact, Langer was the first to observe that the skin is an anisotropic material in the 19th century [26]. He punctured the skin with a circular device and observed that the circular wounds became elliptical in shape when relaxed. Joining the major axes of the ellipses he drew the tension lines of the skin which were named **Langer's lines**. These lines are normally oriented in the direction of the underlying muscles. Later on, the orientation of Langer lines was identified as the preferential orientation of collagen fibers in skin [8, 48], and also it was observed that wounds in the direction of Langer's lines tend

to heal quicker than the ones perpendicular to these lines. Moreover, skin is naturally prestressed [15] which add complexity to its behavior.

The complex multilayered architecture and composition of the skin together with the Langer's lines makes the skin a complex material from a mechanical point of view. Also the variable layer composition and thickness which depends on the individual, body site and age make even more difficult the adoption of a single constitutive model for the healthy skin. Different mechanical models have been adopted to characterize this intrincated mechanical behavior considering its viscoelasticity [27], hyperelasticity [3] and anisotropy [43]. However, the models ranged from the simplest ones which assume the skin as a monolayered linear elastic material, to the most complex which consider skin anisotropy and its multilayered nature [14]. The constitutive model adapted when considering its anisotropy is the same as in other fibered soft tissues [17]. Other characteristics such as the viscoelasticity associated to its multiphasic nature has also be taken into account. Most of the models used for healthy skin are monolayered models; however, recently multilayered models which considered the different behavior of the layer have also be introduced to simulate healthy skin [14].

3 Wound Healing

Wound healing is a dynamical complex organized process which aims at restoring the injured skin. It involves the interplay of different cellular species, extracellular matrix components, biochemical factors such as growth factors and cytokines together with the mechanical environment. The cell-cell and the cell-extracellular matrix interactions are also really important for the success of this process. Wound healing is divided into three phases which are overlapped in time: hemostasis phase, re-epithelization phase and maturation or remodeling phase.

The injury disrupts the tissue and provokes haemorrhage. The first response of the organism to the injury is the formation of a blood clot to reduce blood loss, this is the coagulation or **hemostasis phase**. Mechanical properties of this blood clot are really important for the whole wound healing process, it will serve as a provisional matrix for cell migration. In this phase, platelets play a pivotal role (Table 1) since they reduce blood loss and also release platelet-derived growth factor (PDGF) which will stimulate fibroblast proliferation and chemotaxis [16]. **Inflammation** starts around one hour after the injury, neutrophils migrate to the wound site and remove debris tissue, death cells and bacteria.

In the **proliferative phase** the blood clot is gradually replace by granulation tissue and new blood vessels are developed in the angiogenesis process. Fibroblast and endothelial cells proliferate and migrate to the wound site around three days after the fracture. Endothelial cells start to form new capillarities from existing ones. Fibroblast secrete collagen type I, glycosaminoglycans and proteoglycans which are the main components of the extracellular matrix. This early collagen secretion results in an initial rapid increase in wound strength. Epithelial cells migrate to

Table 1 List of cellular species involved in wound healing

Name	Function	Location	Healing stage/ process	Others
Adipose cells/ Adipocytes	Store energy as fat	Hypodermis		Synthesize several hormones
Endothelial cells	Form new capillaries during angiogenesis	Blood vessels	Epithelialization	Motile cells that migrate to the wound site during the epithelialization stage
Fibroblasts	Synthesize collagen type III Generate contraction forces	Dermis Hypodermis	Epithelialization Contraction	Motile cells that migrate to the wound site during the epithelialization stage
Keratinocytes	Main constituent of the epidermis Form a barrier against environmental damage Modulates the immune system producing anti-inflammatory mediators	Epidermis	Epithelialization	Motile cells that migrate to the wound site during the epithelialization stage
Macrophages	Phagocytosis Eliminate bacteria and dead cells at injured sites	Dermis Hypodermis	Inflammation	Derived from blood monocytes Enter the damaged site through the endothelium of blood vessels Chemotactically attracted to the wound site by cytokines released from damaged cells Part of the immune system
Myofibroblasts	Synthesize collagen type III Generate contraction forces Secrete factors that induce angiogenesis	Dermis	Epithelialization Contraction Angiogenesis	Differentiated fibroblasts Non-motile
Neutrophils	Eliminate bacteria and dead cells at injured sites	Bloodstream	Inflammation	Attracted by inflammation factors Part of the immune system
Platelets	Prevent bleeding forming a plug where there is vascular endothelial damage Blood coagulation	Dermis	Hemostasis	Release PDGF

the wound site and secrete different growth factors such as transforming growth factors α and β (TGF-α, TGF-β) which stimulate wound contraction. Fibroblast and myofibroblast contract the wound reducing its size. Myofibroblasts disappear

after this phase by apoptosis. Nevertheless, the activity of myofibroblasts persists in pathological healing, such as in hypertrophic scars and contractures [21].

Finally, the **remodeling phase** takes place, which could last for years. The collagen type I deposited during the proliferative phase is replaced by collagen type III. This new deposited collagen is reorganized along the Langer's. Once the remodeling phase is concluded, the tensile strength of the scar is about 70% of the tensile strength of the healthy skin about six weeks after injury, which is close to the maximal tensile strength the scar can achieve 75–80% of the healthy skin [29].

All these processes are guided both by biological and mechanical stimulus. The main cellular species involved in healing are summarized in Table 1.

4 Severe Wound Treatments

Different treatments are used when a wound present problems while healing. If the wound is large, it is normally covered by a skin substitute. Even though the autologous skin graft is the preferred technique from a biological point of view, it presents problems of availability and different side effects. Thus, currently the tendency is towards artificial grafts. When the wound is profound, the skin substitute is combined with different pressure therapies in order to accelerate and improve healing.

Skin Scaffolds

Skin substitutes have arisen as a promising technique to treat wounds in which coverage of large areas of skin is required. Indeed, skin substitute engineering is a growing area of research; skin substitutes are widely used in clinics nowadays. In fact, different commercial skin grafts are routinely used in the clinical practice [38].

Artificial skin substitutes provide an immediate protective barrier for the wound. They prevent dehydratation, the entrance of strange particles in the wound (microorganisms and toxins); they also provide an immediate matrix for cell adhesion and revascularization of the wound. As wound healing progresses the scaffolds are integrated in the wound, through the cell migration and vascularization into the skin graft. Sometimes the scaffold is seeded with different cells or growth factor to favor its integration.

Skin substitutes could be classified as dermal, epidermal or dermo-epidermal [53] depending of the area to cover. They could be also biodegradable or non-biodegradable [53]. Regarding their mechanical and structural properties, first skin substitutes (now in the market) were designed to replace healthy skin, they try to mimic the properties of this healthy skin. Nevertheless, there is not a scaffold which provide results comparable to biological skin [38]. Different problems arise in the outcome of wound healing such as poor angiogenesis and scaring of the wound mar-

gins. This is due to the fact that the architecture and composition of the skin scaffolds is much simpler than the one of the healthy skin; for example, skin scaffolds lack of skin appendages (hair follicles, sweat and sebaceous glands) [19] which modify its mechanical properties. These appendages represent discontinuities in the tissues which change skin permeability.

Now the trends go towards skin substitutes which promote wound healing rather than the ones which try to replace skin. Thus, the properties of new scaffolds try to mimic the ones of the wound to allow the different phases of wound healing to happen. Moreover, different cells populations and growth factors are integrated in the new generation scaffolds [66]. Special attention is been paid to the morphology and architecture of the scaffolds at different scales. Since it has been observed that to properly promote regeneration it is really important not only mimic the micro-scale morphology of the ECM of the wound but also its nano-scale morphology [66]. For example a key aspect to promote cell growth is the pore size at different scales. The new research developed to understand cell-cell and cell-ECM interactions [11], have emphasized the need to preserve the properties of the ECM of a normal wound. In this sense, it is also important the use of mixing material better than single materials [10].

Pressure Wound Therapies

Negative pressure wound therapies (NPWT) use a suction device to apply a subatmospheric pressure to the wound, which has been previously filled with a porous foam and sealed with an adhesive. Different interface materials and suction devices have been used since this treatment was first proposed by Morykwas et al. [41].

This treatment is based in mechanotrasduction principles, that is the cells are able to sense the mechanical stimulus and translate it into different biochemical and mechanical signal. This principle was first used to induce regeneration and formation of bone by Ilizarov and Soybelman [23], who first applied traction force in order to generate new bone through distraction osteogenesis. Here, tensile strains are also induced in the wound borders. This deformation stimulates angiogenesis, migration, proliferation and extracellular matrix production. Moreover, the suction removes excess of fluids in the wound. The pressure is applied till the wound is filled by granulation tissue or the wound is prepared for surgery. The level of microdeformation induced in the interface of the wound with the foam dressing depends not only on the applied pressure but also on the foam pore size. In fact, the pore size of the foam is directly related with the amount of granulation tissue formation [20].

The pressure is applied continuously or intermittently. Better results are observed when it is applied intermittently, nevertheless it requieres more care of the patient and it also results in discomfort for the patient [22].

5 Computational Models of Wound Healing

As already mentioned, the healing of a wound involves the coordinated interaction of a wide range of chemical factors and cellular species. Numerous models have tackled the different phases of the healing process, frequently from a continuum point of view. This path was initiated in the early nineties by Sherratt and Murray [51] and Tranquillo and Murray [57], since then it has experienced an increasing attention. First wound healing models were dedicated to the biochemical aspects of the process, mainly focusing on the relation between cellular species and the growth factors that guide the cellular evolution during healing. Sherratt and Murray [51] developed one of the first wound healing models in which they studied the evolution of epidermal cells and a mitosis-regulating growth factor. This model evolved to more complex ones [44, 57] through the inclusion of more cellular species such as fibroblasts and myofibroblasts and the concentration of collagen ECM in the skin. Moreover, these models added the mechanical properties of the skin in order to evaluate the ECM displacement that the skin suffers during wound healing. Most wound healing models that include mechanics are focused on the contraction stage during wound healing [25, 42, 44, 57, 60–65] and one of their main objectives is to quantify the size reduction that the wound suffers during healing. Nevertheless, other models pay attention to other phenomena that occurs during wound healing such as angiogenesis [12, 13, 24, 33, 35, 45, 46, 49, 59, 63, 64]. Most of the angiogenesis works include the oxygen as a main factor in their models together with the influence of angiogenic growth factors.

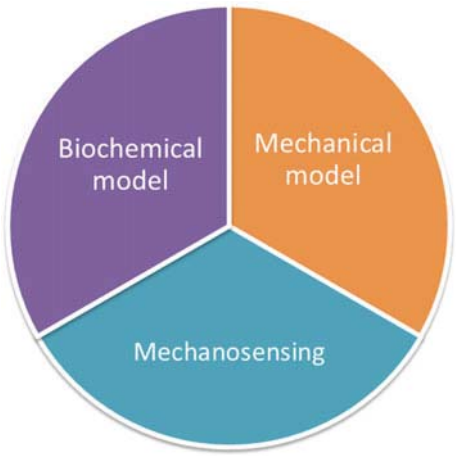

Fig. 1 Scheme of the mechanobiological models. They consist of three main blocks: the biochemical evolution of tissues, the constitutive model of the tissues in which mechanical stimulus is determined and the mechanosensing mechanism of the cells in which the two first block are connected

When the mechanical behavior of skin is included in wound healing models one of the most important aspects is the constitutive material model used to reproduce skin properties. In most wound healing models skin is considered to behave as a viscoelastic material [25, 35, 42, 44, 57, 63–65]. However, it is known that the real behavior of the skin is more complex and in the last years more realistic constitutive material models have been considered. Valero et al. [59, 60] included isotropic hyperelastic skin properties to simulate angiogenesis during wound healing and later added the anisotropic component [62]. In that work, Valero et al. [62] included the effect of fiber anisotropy in three dimensions to reproduce the real collagen fiber network of the skin. Collagen fibers are oriented according to two preferred directions and thus skin behavior is not the same along every direction.

Most of the previous models study wound healing under normal conditions, without assistance. Nevertheless, some models simulate the evolution of wound healing under different therapies that improve or accelerate healing. Lott-Crumpler and Chaudhry [32] analyzed different suture patterns in wounds with complex shapes to find the best resulting stress distribution. Buganza Tepole et al. [7] also used stress analysis to predict healing during the application of skin flaps in reconstructive surgery. Flegg et al. [12, 13] studied the effect of hyperbaric oxygen therapy in wound healing paying attention to the angiogenesis process. They studied the appearance of capillary tips and the evolution of blood vessels according to the oxygen supply.

General Framework of Continuum Wound Healing Models

The pioneering models, and the more recent ones, are built upon a set of coupled diffusion-convection-reaction equations, one for each species in the model. The considered species represent the averaged values of their biological counterpart, such as endothelial cells, fibroblasts or myofibroblast densities (i.e. number of cells per unit of volume), extracellular matrix fibers such as collagen (in its different types), fibrin and cytokines and growth factor concentrations (i.e. mass per unit of volume) such as VEGF, TFG-β. The system of equations to be solved is frequently non-linear (to cope with the intricate communication between cells, cell-receptors and mechano-biological cues) and strongly coupled (to cope with the cascade of events that lead the activity of a certain cellular species triggered by precursor factors). The governing equations ruling these models are formulated attending to the conservation of cellular and chemical species and the conservation of tissue momentum, together with the mechanosensing mechanism of the cell (Fig. 1). This mechanosensing of the cells is represented through their capacity to sense their mechanical environment and translate it to regulate the forces that they exert on the tissue and also to regulate other biological processes such us differentiation and extracellular matrix production. The use of non-coupled models between biochemical and mechanical processes allow to deal with the different time scales at which biochemical and mechanical event occurs at the wound site.

Thus, if we consider a system with n cellular or chemical species c_i (with $i = 1, \ldots, n$), the conservation of the first one, c_1, can be formulated in general terms as

$$\frac{\partial c_1}{\partial t} + \nabla \cdot \mathbf{J}_1(t, \mathbf{x}, c_1, \ldots, c_n, \mathbf{u}) = f_1(t, \mathbf{x}, c_1, \ldots, c_n, \mathbf{u}, \boldsymbol{\sigma}) \tag{1}$$

where t denotes the temporal variable, $\mathbf{x}$ the spatial coordinate, $\mathbf{J}_1$ denotes the net flux of species c_1 and f_1 denotes the net production of species c_1. In this general framework, the flux term $\mathbf{J}_1$ may account for factors such as random migration, biased migration (chemotaxis, haptotaxis and/or durotaxis) and the passive drag of species due to the deformation of the host tissue. This can be mathematically formulated as

$$\begin{aligned}\mathbf{J}_1(t, \mathbf{x}, c_1, \ldots, c_n, \mathbf{u}, \boldsymbol{\sigma}) = &- D_1 \nabla c_1 + \frac{a_{12}}{(A_{12} + c_2)^2} c_1 \nabla c_2 + a_{13} c_1 \nabla c_3 \\ &+ a_{1,cell} \nabla \cdot \boldsymbol{\sigma}_{cell} + c_1 \frac{\partial \mathbf{u}}{\partial t},\end{aligned} \tag{2}$$

where the first term of Eq. (2) accounts for the random migration (or diffusion) of c_1, the second and third terms account for the biased migration upwards a chemotactic factor c_2 (chemotaxis) and a ECM fiber density c_3 (haptotaxis), the fourth term accounts for the biased migration towards stiffer or strained regions (durotaxis/tensotaxis), and the last term accounts for the passive drag of substance c_1 due to the extracellular matrix deformation (represented here by the displacement field $\mathbf{u}$ and the subsequent velocity field $\frac{\partial \mathbf{u}}{\partial t}$). The chemotactic sensitivity function $\frac{a_{12}}{(A_{12}+c_2)^2}$ is taken from Olsen et al. [44] based on Michaelis-Menten reaction kinetics between the chemotactic factor and the receptors at the cell surface [52]. The haptotactic term, $a_{13} c_1 \nabla c_3$, is taken from Tao and Winkler [55], whereas the durotaxis term, $a_{1,cell} \nabla \cdot \boldsymbol{\sigma}_{cell}$, is taken from Moreo et al. [40]. Note as well that the durotaxis term, $a_{1,cell} \nabla \cdot \boldsymbol{\sigma}_{cell}$, includes implicitly the cell density, c_1, through the definition of the cell induced stress, $\boldsymbol{\sigma}_{cell}$, as shall be detailed below. In both cases the sensitivity coefficient (a_{13} and $a_{1,cell}$) is considered constant.

Similarly, the net production of species c_1 can present an expression of the form:

$$\begin{aligned}f_1(t, x, c_1, \ldots, c_n, \mathbf{u}, \boldsymbol{\sigma}) = &\left(r_1 + \frac{r_{12,max} c_2}{C_{12} + c_2}\right) c_1 \left(1 - \frac{c_1}{K_1}\right) - \frac{k_{14} c_2}{K_{14} + c_2} \theta^+ c_1 \\ &+ k_{41} c_4.\end{aligned} \tag{3}$$

This net production expression takes into account the proliferation of species c_1 and the differentiation of species c_1 into species c_4, both mechanisms upregulated by the mitotic factor c_2 (through a saturation-like kinetics of the form $\frac{K_{14} c_2}{K_{14}+c_2}$, where K_{14} denotes the maximum stimulus prompt by the cytokine c_2 and K_{14} regulates the influence of cytokine). Moreover, the differentiation from c_1 to c_4 includes as well the influence of tissue stiffness and cell stretch (Fig. 2) through the term $\theta^+ = \max(\theta, 0)$

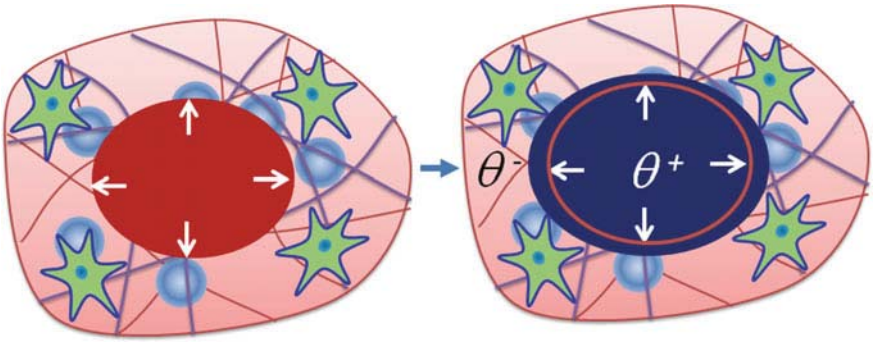

Fig. 2 Initial mechanical environment of the injured site. After the injury takes place it is distracted due to the prestresses of the healthy skin that make volumetric strains in the wound to be positive and negative in the healthy skin. This distribution of stresses will induce differentiation of fibroblast into myofibroblasts inside the wound

(where $\theta = \nabla \cdot \mathbf{u}$) [60]. Finally, the last term on Eq. (3) accounts from the backward differentiation from species c_4 to species c_1, modeled through a linear kinetics.

The conservation of tissue momentum can be written in general form as

$$\nabla \cdot (\boldsymbol{\sigma}_{ecm} + \boldsymbol{\sigma}_{cell}) = \mathbf{f}_{ext}, \tag{4}$$

where $\boldsymbol{\sigma}_{ecm}$ denotes the host tissue constitutive material law (which can chose among elastic, viscoelastic, hyperelastic, with or without anysotropic effects [62]), $\boldsymbol{\sigma}_{cell}$ denotes the cell-induced stresses and $\mathbf{f}_{ext}$ the external forces acting on tissue. Normally, the external forces are assumed to be linear with the tissue stiffness, modeled as the product of cell density and extracellular matrix fibers density. The cell-induced stresses $\boldsymbol{\sigma}_{cell}$ is more accurately represented as the product of the net force per a single cell and the cellular densities exerting traction forces, as for instance

$$\boldsymbol{\sigma}_{cell} = p_{cell}(\theta) c_1 \mathbf{I} \tag{5}$$

where an isotropic behavior has been assumed, governed by the cellular function $p_{cell}(\theta)$ that copes with the regulation of the traction force by the tissue stiffness through the cellular volumetric deformation θ [60].

With this model different results could be obtained such as the temporal evolution of the different cellular and chemical species and the extracellular matrix mechanical environment (Fig. 3). Models as the above-described have been extensively used to analyze the course of the healing process, and in particular, to unveil patterns leading to unsuccessful healing. Most attention has been driven to hypoxic wounds, where an insufficient oxygen supply impedes the progress of healing. In this line, Flegg et al. [12] used a biochemical model of angiogenesis to investigate the impact of oxygen based therapies. More extensive models of angiogenesis include the effect of the tissue mechanics, such as Schugart et al. [49] and Xue et al. [65]. Menon et

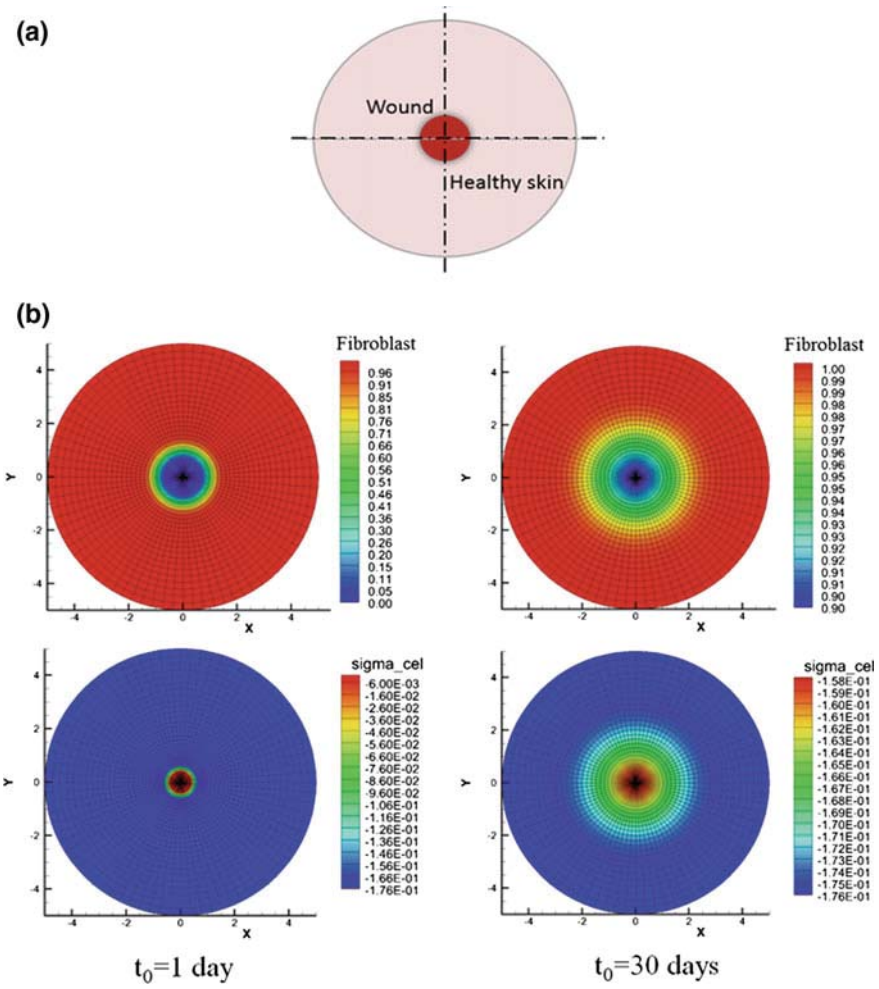

Fig. 3 **a** Superficial circular wound simulated in which both wound and surrounding healthy skin are included; **b** evolution of fibroblast inside the wound and volumetric stress (MPa). The model proposed in [59] has been used in the simulation. Note the different scales used in the contour legends at the begin and end of the simulation

al. [37] used a biochemical model of wound closure to study the effect of prolonged inflammation on the healing kinetics. Purely mechanical models have been also considered to design improved cushions for preventing pressure ulcers in patients with reduced motility [30].

6 Discussion and Conclusions

In silico models represent a powerful tool to understand the wound healing process and how different factors influence its outcome. Note that they offer a straightforward framework to isolate not only the mechanical variables but also the biochemical ones. Besides, physically based models set a step forward in the understanding of different phenomena such as the differentiation of fibroblast into myofibroblast and the contraction kinematics [60]. Thus, *in silico* models provide a cheap platform on which elucidate the main variables which will be important to produce future skin substitutes which better mimic the morphology of the wound and skin. Moreover, they add the possibility of performing patient and site specific simulations in order to determine the viability of different treatments before their application; as well as the opportunity to simulate both physiological and pathological states.

Some ideas arise when analyzing the results of computational models of wound healing, regarding the design of new treatments. First, the design of patient specific skin substitutes could aid to reduce scarring by controlling tissue contraction, in this respect, wound morphology could be naturally incorporated. Secondly, the skin scaffold need to adequately mimic the material properties of the substituted tissue (such as stiffness evolution, anisotropy, prestress and Langer's lines). Similarly, the use of negative pressure therapies is conditioned to a better knowledge of the pressure level to apply. Thirdly, it is equally important to take into account the interaction of cells with the skin substitutes by mechanosensing and how cell-cell interactions are modified when the tissue properties are not the same as the wound or the healthy tissues. And last but not least, a deeper knowledge of the intricate governing the healing cascade need to be gained before a definitive wound healing treatment for severe wounds can be found. As far as we know, the complete aetiology of this injuries is not completely understand, and, despite the efforts made to treat and prevent severe wounds their prevalence rate remains still high.

The design of skin substitutes has been traditionally made with passive and biologically compatible scaffolds which replace healthy skin. However, the new trend goes towards skin substitutes which mimic the mechanical and architectural properties of the wound [39] in order to actively promote healing through mechanotransduction. Cells detect, react and adapt to the mechanical stimulus that they feel, which is undoubtedly influenced by the mechanical properties of the skin and the wound along the healing process. This aspect should be considered when designing new treatments. It may be important to properly reproduce the mechanical environment in each time point of the healing process in order to better reproduce the environment which better induce cell activity and produce no alterations in cell mechanotransduction when compared with normal wound healing. This will be essential to understand mechanotransduction of cells and how it could be altered due to different extracellular matrix environments such as the ones of skin substitutes. *In silico* models could

also be used to determine the outcome of each skin substitute before implantation. Moreover, different new factors should be incorporated in *in silico* models.

The importance of considering the different spatial and temporal scales that characterize the healing process has been proven experimentally through the study of various treatments [11, 20, 66]. It is necessary to go down until the nanoscale to understand the cell-to-cell and cell-to-ECM interactions that guide the healing process. Different attempts have been made to reproduce this interaction such as the proposal of cellular function laws based on experimental evidences [60]. However, in order to properly determine the interaction of the cell and extracellular matrix, new models of wound healing should incorporate the nanoscale implicitly. Recently, several models of single cell events such as migration of cells in two [40] and three dimensions [4, 5] have been developed. However, to completely understand the aetiology of severe wounds (and consequently search for the most suitable treatment) it is necessary to incorporate all the involved scales in order to unveil the individual role of each factor at each scale and the interactions among scales. Moreover, as far as we know, the topology and internal architecture of the wounds has not been incorporated in the models, even though they guide different events of healing [20, 66].

Additionally, it would be also very important to understand the way skin develops during embryogenesis and fetal growth. Computational models focused on these early phases could also aid in understanding the the healing process, as well as exploiting their complementary nature respect to in vivo and in vitro experiments an their intrinsic capability of individuating the role of mechanics on it. This would guide the design of more complex structures for skin scaffolds [38] as well as the analisys of more favorable mechanical environments for skin development and healing. The absence of scaring in fetal wounds [28] could be considered as the ideal situation to be mimicked.

Finally, it should be noticed that a wide range of approaches have been developed to study wound healing, both from experimental and computational perspectives. However, these approaches are closed to the one specific aspect under consideration. It has been proved for other biological tissues [18] that the interrelationship among the different events can have and important role in the improvement of healing and consequently the design of new treatments. Therefore, it seems more convenient nowadays to exploit the specific knowledge in the last decades in a joint and global model of the healing process.

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