



A Cell-Regulatory Mechanism Involving Feedback between Contraction and Tissue Formation Guides Wound Healing Progression

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Abstract

Wound healing is a process driven by cells. The ability of cells to sense mechanical stimuli from the extracellular matrix that surrounds them is used to regulate the forces that cells exert on the tissue. Stresses exerted by cells play a central role in wound contraction and have been broadly modelled. Traditionally, these stresses are assumed to be dependent on variables such as the extracellular matrix and cell or collagen densities. However, we postulate that cells are able to regulate the healing process through a mechanosensing mechanism regulated by the contraction that they exert. We propose that cells adjust the contraction level to determine the tissue functions regulating all main activities, such as proliferation, differentiation and matrix production. Hence, a closed-regulatory feedback loop is proposed between contraction and tissue formation. The model consists of a system of partial differential equations that simulates the evolution of fibroblasts, myofibroblasts, collagen and a generic growth factor, as well as the deformation of the extracellular matrix. This model is able to predict the wound healing outcome without requiring the addition of phenomenological laws to describe the time-dependent contraction evolution. We have reproduced two *in vivo* experiments to evaluate the predictive capacity of the model, and we conclude that there is feedback between the level of cell contraction and the tissue regenerated in the wound.

Citation: Valero C, Javierre E, García-Aznar JM, Gómez-Benito MJ (2014) A Cell-Regulatory Mechanism Involving Feedback between Contraction and Tissue Formation Guides Wound Healing Progression. PLoS ONE 9(3): e92774. doi:10.1371/journal.pone.0092774

Editor: Adam J. Engler, University of California, San Diego, United States of America

Received: October 7, 2013; **Accepted:** February 25, 2014; **Published:** March 28, 2014

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Funding: This research was supported by the Spanish Ministry of Economy and Competitiveness (Grant DPI2012-32880 and BES2010-037281) (http://www.mineco.gob.es/porta/site/mineco/?lang_chosen=en). Financial support of the European Research Council (ERC) through project ERC-2012-StG 306751 is gratefully acknowledged (<http://erc.europa.eu/>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Wound healing is an intricate process that combines biological, chemical and mechanical signals for collective cell function. Normal wound healing evolves over three overlapping phases: inflammation, proliferation and remodeling [1,2]. When homeostasis is reached a few hours after wounding, the inflammatory phase begins with neutrophil and macrophage cell invasion and debridement of the wound site [1]. Subsequently, these cell types secrete and/or recruit specialized biochemical growth factors, such as TGF- β , PDGF and MDGF which control the subsequent stages of the healing process. Re-epithelialization of the wound also occurs during the inflammation phase. Epithelial cells proliferate and move to the top of the wound. During the proliferative phase, biochemical mediators recruited during the inflammatory phase control the migration, proliferation and bio-signal production of fibroblasts and endothelial cells. Fibroblasts degrade the initial fibrin blood clot [3] and secrete collagen type III, creating a new extracellular matrix at the wound site that is more resistant than the blood clot but has inferior mechanical properties than the undamaged tissue. The inferior mechanical properties of the granulation tissue are due to, among other factors, the random alignment of the new secreted collagen fibers.

Matrix remodeling occurs over a period of months, increasing the proportion of collagen type I and causing the formation of scar tissue that resembles healthy skin. Endothelial cells follow migrating fibroblasts and re-establish the vascular system that provides the oxygen and nutrients required for cell function. There is evidence that both biochemical factors (such as TGF- β) [4] and mechanical stimuli induce the differentiation of fibroblasts into myofibroblasts [5], leading to wound contraction.

Tissue cells are anchored to a substrate and use their actomyosin system to exert and transmit contractile forces to their surroundings [6]. Mechanical stimuli are known to influence several cellular processes such as migration, differentiation and orientation [7–12]. Moreover, there is evidence that the mechanical stimulus that regulates these processes is the stiffness of the substrate that surrounds the cells [5,8,13–17]. To clarify this phenomenon, Mitrossilis et al. [15] demonstrated that cells on elastic substrates modify their activity according to the substrate stiffness. Their *in-vitro* experiments demonstrated that the forces exerted by cells increase as the substrate becomes stiffer [15], and that a saturation force level is reached. Cells are anchored to the substrate through focal adhesions and show different behaviors depending on the mechanical properties of the substrate; they are stronger on stiffer surfaces.

Computational modeling makes it possible to reproduce and evaluate the wound healing progress under different conditions. To provide valuable predictions, the healing process needs to be fully understood and translated into mathematical equations. Moreover, computational models can be of great aid for the discussion of certain biological hypotheses. Early wound healing models [18–20] could predict the evolution of epidermal wounds. Murray et al. [19] developed the first biochemical model of wound contraction in one dimension, which was used to study the evolution of a cellular species and the extracellular matrix (ECM) density and displacement. Sherratt et al. [20] proposed a biochemical model in which cell proliferation and migration are dictated by a generic growth factor. These models have been further developed to incorporate biophysical evidence acquired from *in vitro* or animal models. Olsen et al. [21,22] proposed the first mechano-chemical model of wound contraction, in which the major events in fibroplasia and wound contraction are taken into account, including the addition of a new cellular species under study, the myofibroblasts, which have a relevant role in wound contraction. A thorough analysis of these model equations enabled the establishment of the effect of chemical net production on the occurrence of fibroproliferative disorders, particularly the effect of a permanently contracted state. Adam [18] investigated the occurrence of non-healing wounds and the so-called critical size defect with a simple model that describes the evolution of a generic growth factor activating cell proliferation at the wound edge. Olsen's model [21] has been recently revised by different authors [23–25]. These works incorporate the decreased mechanical properties of the granulation tissue and combine for the first time the coupled actions of chemical and mechanical factors on the fibroblast to induce myofibroblast differentiation, although the studies differ in the mechanical stimulus used to drive the differentiation. Both works suggest that differentiation is guided by stress. Whereas Javierre et al. [23] claim that the stress that guides the process is the force exerted by the cells, Murphy et al. [24] propose that this stress comes from the elastic component of the ECM. Additionally, Javierre et al. [23] investigated the effect of wound shape on the contraction kinetics, whereas Murphy et al. [24,25] focused on a more detailed representation of the biochemical signaling of wound contraction. Furthermore, Javierre et al. [23] considered a unique growth factor that regulates differentiation and collagen production, whereas Murphy et al. [25] included the chemical kinetics of two different growth factors (PDGF and TGF- β) separately.

Several cellular mechanisms have been found to be driven by the stiffness of the substrate that surrounds the cells and not by the stresses that the cells support [8,15]. Thus, we propose a differentiation mechanism that combines both chemical factors and a mechanical stimulus, as performed in previous works, but we assume that the mechanical stimulus that regulates the differentiation process is the ECM deformation, which depends directly on the ECM stiffness.

Therefore, in this work, we propose a unified constitutive theory consistent with experimental observations of individual and collective cell populations. This theory is based on a rigidity sensing mechanism that cells use to control the level of contraction that they exert on the ECM to drive its deformation. This deformation of cells is able to indirectly regulate the progression of different cellular events, such as cell differentiation and tissue formation.

Results

Cell traction forces are modulated in response to the rigidity of the surrounding ECM

Early works on wound contraction assumed that cells exert a constant traction force (denoted by τ_0 or λ) on the ECM. This constant traction force is subsequently scaled (or modulated) by the ECM density (ρ) and the cellular densities of fibroblasts (n) and myofibroblasts (m). Traditionally, a linear relationship between cell-induced stresses and cell densities is assumed. Moreover, the myofibroblasts-enhanced traction forces are modeled through the proportionality factor ξ . Finally, the most significant difference between these models arises in the term for the ECM density. This term represents the different properties of the involved tissues (the wound and the partly recovered and healthy skin) during contraction progression. All of these hypotheses have been included through different phenomenological laws, such as [21,23]

$$\sigma_{cell} = \tau_0 \frac{\rho}{R_t^2 + \rho^2} (1 + \xi m) n \mathbf{I} \quad (1)$$

or [25]

$$\sigma_{cell} = \lambda \rho (n + \xi m) \mathbf{I} \quad (2)$$

These expressions aim to induce an increase in the cell-exerted stresses in the middle of the wound, creating a stress gradient between the wound and the surrounding healthy tissue. Note that this gradient is therefore dependent on the initial conditions of the model with respect to the ECM density and the cell populations.

Other authors instead proposed phenomenological laws that are non-linear to the cell population. In those cases, the stresses exerted by cells tend to become saturated due to contact inhibition and competition for ECM binding sites at high cell densities [26]

$$\sigma_{cell} = \tau_0 \frac{\rho}{1 + \gamma n^2} n \mathbf{I} \quad (3)$$

However, there are multiple experiments that suggest that the cellular capacity to exert traction forces on the ECM strongly depends on the ECM stiffness [27]. Therefore, in this work we propose a purely mechanical and self-regulated traction force dependent on the ECM stiffness through

$$\sigma_{cell} = p_{cell}(\theta) (n + \xi m) \mathbf{I} \quad (4)$$

In this expression, we consider the role of the ECM stiffness through p_{cell} , which denotes the force that a cell exerts depending on the volumetric strain (θ) of the ECM [28]. Unlike other models, we do not explicitly include the ECM density in the expression for σ_{cell} . However, the ECM density does play an indirect role in cell-induced stresses through the value of p_{cell} (see File S1). As the collagen density increases, the tissue becomes stiffer [29], regulating the volumetric strain of the tissue (θ), which in turn defines the value of p_{cell} and the stresses exerted by the cells on the ECM. We consider also that σ_{cell} depends on the concentration of fibroblasts and myofibroblasts, with a term $(n + \xi m)$ similar to the one proposed by Murphy et al. [25]. Contractile forces exerted by fibroblasts can initiate wound closure and myofibroblasts are

known to contribute to the transmission of these contraction forces [29,30]. Furthermore, any of these species can be present in the absence of the other. Hence, when one of them is not present, the other one can still generate stress [31]. This basic effect is not included in most of the previous models, in which myofibroblasts are not considered [26] or in which the generated forces are always zero in the absence of fibroblasts [21,23].

Figure 1 shows that the initial stress distributions (σ_{cell}) exerted by the cells in the wounded and unwounded tissues are very similar in all considered theories. Therefore, we can conclude that all previous phenomenological laws represent similar behavior and thus, we can find a clear biophysical interpretation of this behavior which is the mechanosensing mechanism provided by cells.

Fibroblast to myofibroblast differentiation is driven by cell deformation

We assume that fibroblasts differentiate into myofibroblasts in response to the strain (θ_{cell}) supported by the fibroblasts. This strain is the same as the strain of the ECM because we consider cells and the ECM to occupy the same domain and because they both support the same strain as the compatibility condition.

This assumption for fibroblast differentiation into myofibroblasts is based on the following phenomena. When a wound occurs and healing events are activated, fibroblasts exert contractile forces as a mechanosensing mechanism. Thus, fibroblasts shrink the external domain of the wound, and consequently, the inner part of the wound is extended. This effect forces the fibroblasts inside the wound to stretch. To overcome this effect, we hypothesize that fibroblasts differentiate into myofibroblasts regulated by the passive stretching that fibroblasts support inside the wound due to the fibroblast contraction in the external part of the wound. This result is consistent with experimental evidence that establishes that mechanical forces such as stretching can drive fibroblasts to differentiate toward a myofibroblast phenotype [29,32,33]. When the population of myofibroblasts inside the wound also exerts contractile stresses, the full contraction of the wound occurs, and the differentiation of fibroblasts to myofibroblasts is stopped.

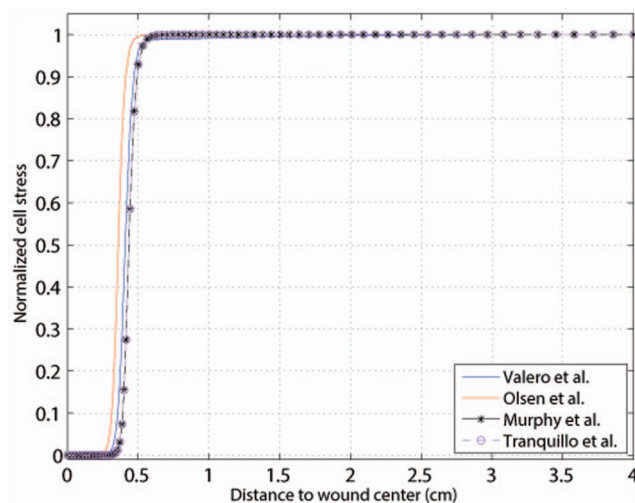


Figure 1. Normalized cell stress (σ_{cell}) distributions created by different laws [21,25,26] and the proposed model at the beginning of wound contraction. The wound has a radius of 0.5 cm. Every law produces a similar stress distribution despite dependence on different variables. doi:10.1371/journal.pone.0092774.g001

Therefore, the first observable consequence of an injury is the distraction of the wound due to fibroblasts distribution (Figure 2). This deformation causes the ECM volumetric strain, θ , to be positive at the wound center and negative (although very close to zero) in the surrounding undamaged tissue (Figure 3), which in turn causes myofibroblasts to appear inside the wound, close to its edge (Figure 4).

As time passes, fibroblasts and myofibroblasts accumulate inside the wound, creating the necessary traction forces to overcome the passive stretch of the wound. From that moment on, the wound contracts, and the sign of ECM volumetric deformation gradually changes from positive to negative from the wound boundary inward (see Figure 3).

Therefore, there are two different behaviors in the wound caused by the non-uniform cell and matrix densities. Cells can deform, contracting the matrix, or cells can be stretched due to the matrix deformation. Therefore, fibroblasts contract the ECM near the wound edge and stretch the wound center. This stretching is also included in the fibroblasts that are inside the wound site, and it regulates their differentiation into myofibroblasts (see Figure 4).

Thus, the proposed differentiation mechanism implies that there is no differentiation from fibroblasts to myofibroblasts in the healthy skin. This outcome is physically coherent, as myofibroblasts appear only inside the wound [31]. The use of volumetric tensile strains to differentiate provides a biophysical explanation for a phenomenon that has been previously simulated in a phenomenological way [21,23,25,26].

Comparative analysis of the predictive ability of the model with in-vivo experiments

The proposed mechano-chemical model makes it possible to study the evolution of the wound from two different perspectives. First, we analyze the deformation of the wound during its contraction. However, the contraction of the wound is accompanied by the synthesis and deposition of new tissue, which fills the wound space. Hence, we also analyze the healing of the wound in terms of collagen density. Collagen does not fill the wound completely until several months or years have passed [1]. Hence, we consider the wound to be healed when its collagen density is at least 75% of the density in healthy skin. It is safe to assume that when this threshold of collagen concentration is reached, the skin has mostly recovered its mechanical properties and functionality.

We have reproduced the wound geometries used by Roy et al. [34] and McGrath and Simon [35] in animal models. Roy et al. [34] considered a circular wound of area of 0.5cm^2 in pigs, whereas McGrath and Simon [35] considered square wounds with areas of 6.25cm^2 and 12.54cm^2 in rats. The area of the tissue initially occupied by the wound is used to determine the contraction pattern of the considered geometries. The temporal evolution of this area (normalized with respect to its initial size) is presented in Figure 6. The release of the skin stresses is a direct consequence of the injury, which causes a fast increase in the wound area. However, as time passes, the wound contracts due to the forces exerted by the cells, finally a size similar to the initial size. Based on the considered geometries, we can conclude that a larger wound size leads to a smoother transition between the distraction-contraction regimes.

The healing pattern for the considered geometries is obtained via the temporal evolution of the wound area (normalized to its initial size). As introduced before, we consider the wound to be all parts of the tissue with less than 75% of the collagen density of the undamaged tissue. This variable is presented in Figures 7 and 8, for the experimental results [34,35]. In both cases, we accurately

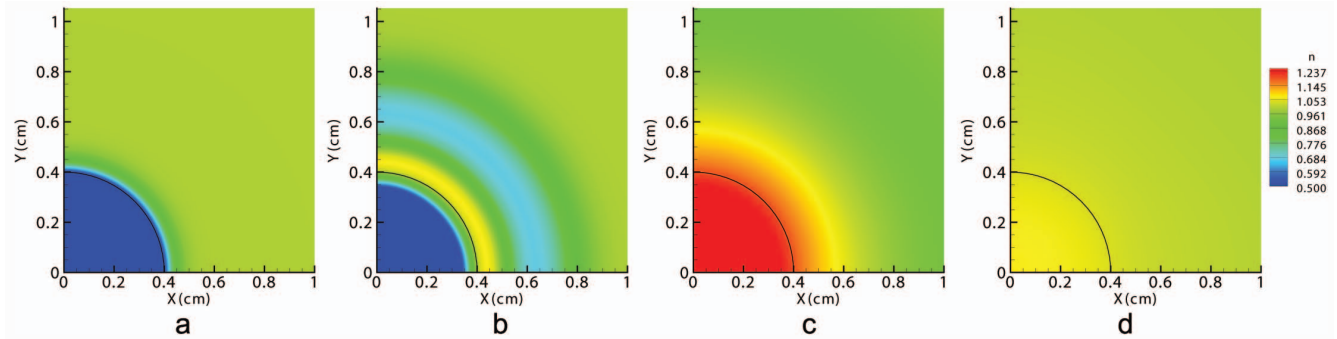


Figure 2. Fibroblast distributions in the tissue at $t=0$ (a), at the beginning of the contraction (b), at halfway through the healing time (c) and at healing time (d). The black line denotes the edge between the initial wound and the surrounding skin.
doi:10.1371/journal.pone.0092774.g002

capture the healing kinetics at the early stages of the healing process. Collagen appears rapidly at the early stages of healing and it stabilizes after reaching its maximum value of wound closure (Figure 5).

Roy et al. [34] observed the evolution of an ischemic wound in a pig. We have used the non-ischemic wound for comparison with our results as we simulate a wound in non-pathological skin. We present wound closure as a function of time. We simulate wound healing in humans, and the experiments were performed on different animal species. When comparing the results, it should be taken into consideration that each species has different time parameters due to their different cellular and tissue kinetics [36]. Hence, for each set of experiments (simulations and in vivo) and in order to adjust the differences in time scales, we fix the healing time as the moment when the maximum healing is reached. For the small circular geometry (area of 0.5cm^2) analyzed by Roy et al. [34] (Figure 7) the numerical simulation closely predicts the closure rate at the latter stages of the healing process. We see that the initial distraction stage lasts for approximately 10% of the healing time and that the healing curves in both cases follow a similar pattern, reaching a similar healing level. In both cases, almost complete healing is obtained.

For the larger geometries, we simulate the experiments of McGrath and Simon [35] (Figure 8), in which square wounds of different areas were considered (sizes of 6.25cm^2 and 12.54cm^2). We observe that the numerical simulation underestimates the percentage of wound closure (for the time period considered). As in the experimental work, we found that the larger wounds heal slightly less than smaller wounds. We also found that the elastic

modulus of the rat's skin is one order of magnitude smaller than the elastic modulus of the pig's skin.

The results show differences between the two cases based on several reasons. First, the mechanical properties of the two animal species have different orders of magnitude. Moreover, the wound sizes should be considered to be of different orders of magnitude in the two experimental works. Although the wound studied by [34] can be considered small relative to the animal size, the wound studied by [35] has a large size compared with the animal size. This fact greatly influences the healing process. In [35] the wound probably affects the muscular zone with movement, which impairs greater healing.

When studying square wounds, we find that the healing pattern tends to soften the curvature of the wound. This phenomenon has been previously observed in other biological processes, such as bone ingrowth in bone scaffolds. This phenomenon corroborates the idea that wound healing is a mechanically driven process [37].

Discussion

The economic and social impact of the treatment of chronic wounds calls for an integrated and multidisciplinary approach to the problem. Mathematical modeling and computer simulation should be used as additional tools to gain a better understanding of the intricate biochemical and mechanical processes behind wound healing.

In this work, we present a mechano-chemical wound healing model with two main novelties that distinguish it from previous models. We postulate that the main phenomena that occur during wound healing involves cells and are regulated by mechanical

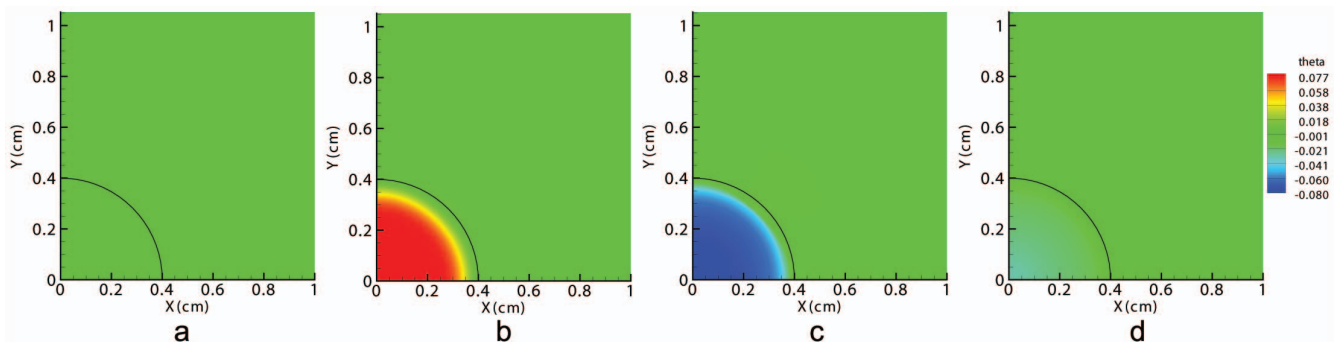


Figure 3. Volumetric deformations in the tissue at $t=0$ (a), at the beginning of the contraction (b), at halfway through the healing time (c) and at healing time (d). The black line denotes the edge between the initial wound and the surrounding skin.
doi:10.1371/journal.pone.0092774.g003

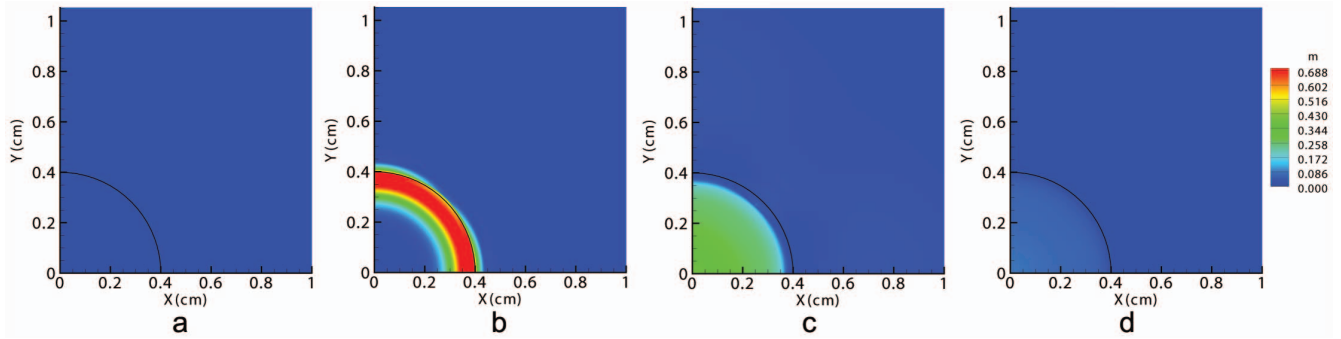


Figure 4. Myofibroblasts distributions in the tissue at $t=0$ (a), at the beginning of the contraction (b), at halfway through the healing time (c) and at healing time (d). The black line denotes the edge between the initial wound and the surrounding skin.
doi:10.1371/journal.pone.0092774.g004

stimulation. Thus, we propose to update the phenomenological laws with physical evidence-based laws for fibroblast differentiation and the cell-exerted stresses.

This work provides a mechanical theory of wound contraction that is consistent with the cell function experimental observations [15,16] and with wound healing in animal models [34,35]. The proposed model generalizes from previous models [19,21–23,25] with a cell-regulatory mechanism that handles ECM rigidization and its impact on cell function. Our model provides similar results to those of previous works, but we have proposed a formulation of cell traction generation and fibroblasts differentiation based on a biophysical hypothesis instead of a phenomenological assumption. Taking these modifications into account, the model can help to clarify our knowledge of regenerative phenomena.

The effect of additional phenomena (naturally produced by the organism or externally induced) could be analyzed with the current model definition, either changing the mechanical properties of the affected tissues to the ones measured for each pathology or varying specific model parameters. This is the case of certain pharmacological therapies [38] or genetic mutations involving modifications in the tissue properties, mostly rigidization, that could be studied using the present model. Moreover, it will be possible the study of certain pathologies such as pressure ulcers [39] or fibroproliferative disorders [40], which have a high mechanical component and modify the natural evolution of wound healing. In pressure ulcers, oxygen flow is impaired due to an excessive pressure in the tissue that comprises blood vessels [39]. Moreover, the hydrostatic pressure becomes negative in the skin area subjected to pressure leading to negative volumetric strains. Thus, fibroblasts differentiation into myofibroblasts will be

inhibited once the ulcer has begun, and traction forces generated by fibroblasts will not be enough for closing the wound. The opposite cases are fibroproliferative disorders such as keloids and hypertrophic scars, which appear due to an excessive collagen production during healing [40]. It is also known that the appearance of these disorders is promoted by mechanical forces [41,42]. An overexpression of collagen will cause an excessive tension in the tissue that surrounds the wound, which will also produce an excessive fibroblast differentiation. Moreover, it is known that the collagen type created in every process is different, having different stiffness properties, which could be included in the model.

Several assumptions and simplifications were needed to formulate and implement this model. First, although wound healing is a three-dimensional process, we considered a two-dimensional simplification in our work. We considered a plane stress approach, neglecting wound depth and assuming that the deformation on the plane is constant.

Most of the existing models [19,21,25] make great simplifications about wound geometry, considering only one-dimensional axisymmetric wounds. This simplification is useful for analyzing theoretical wounds but cannot be applied to simulate real and more complicated wounds. Thus, we follow [23] and consider a two-dimensional model that could be extended to three dimensions, which would be more appropriate to reproduce the real behavior of wounds in the skin.

Other simplifications in the model are made when defining cell stresses. Although we have assumed that stresses are mainly due to the cell activity, other sources such as patient motion could generate stress. We have also considered the volumetric cell strain

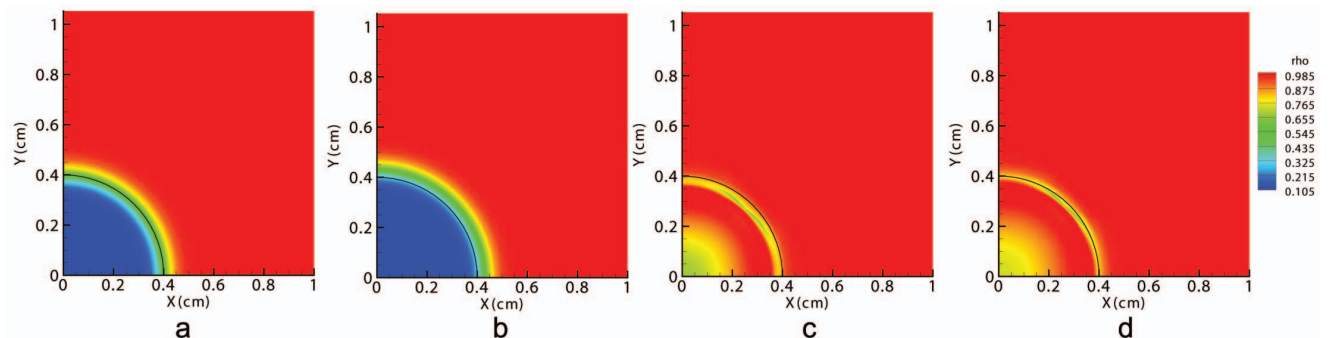


Figure 5. Collagen distributions in the tissue at $t=0$ (a), at the beginning of the contraction (b), at halfway through the healing time (c) and at healing time (d). The black line denotes the edge between the initial wound and the surrounding skin.
doi:10.1371/journal.pone.0092774.g005

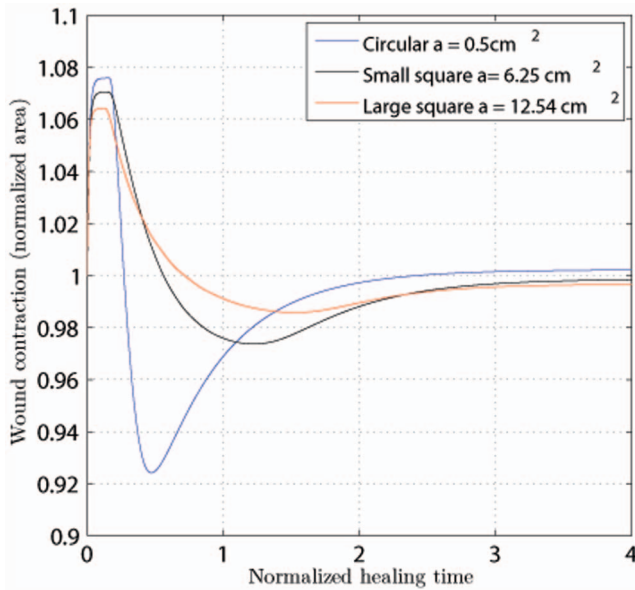


Figure 6. Wound contraction as a function of time for the three studied geometries.
doi:10.1371/journal.pone.0092774.g006

as the mechanical variable that regulates cell biology, however, other mechanical variable such as the deviatoric cell strain or the principal cell strain could have been considered. Moreover, the influences of other factors such as chemical growth factors are indirectly included in the model through the cellular kinetics.

Regarding the numerical results, the wound does not reach complete healing during the studied time. In fact, complete healing is never achieved after a wound occurs [1]; the tissue keeps recovering for months or years. Moreover, a critical size defect (CSD) is known to exist [18]. This CSD is different for each animal species and denotes the wound size above which a wound will not heal during the animal's lifetime.

Although computational simulations reproduce an ideal situation, there are several external and unpredictable factors in animal experiments that should be considered. Moreover, the mechanical properties of the skin vary depending on the location on the body. The skin can displace and contract in different ways depending on how it is oriented relative to tension lines.

Scarring is the step that follows wound contraction, and the model presented here will be of great aid for preliminary qualitative prediction of the scarring level. Moreover, the model makes it possible to study different factors that regulate scarring, such as wound size and shape, the animal species and the mechanical properties of the skin.

Materials and Methods

In this work, we model the temporal evolution of different cellular species (fibroblasts and myofibroblasts), chemicals (a generic growth factor with the combined effects of PDGF and TGF-β on (myo)fibroblasts and the collagen density) and extracellular matrix deformation [30,43]. The cellular and chemical species densities are obtained from a conservation law

$$\frac{\partial Q}{\partial t} + \nabla \cdot \mathbf{J}_Q = f_Q \tag{5}$$

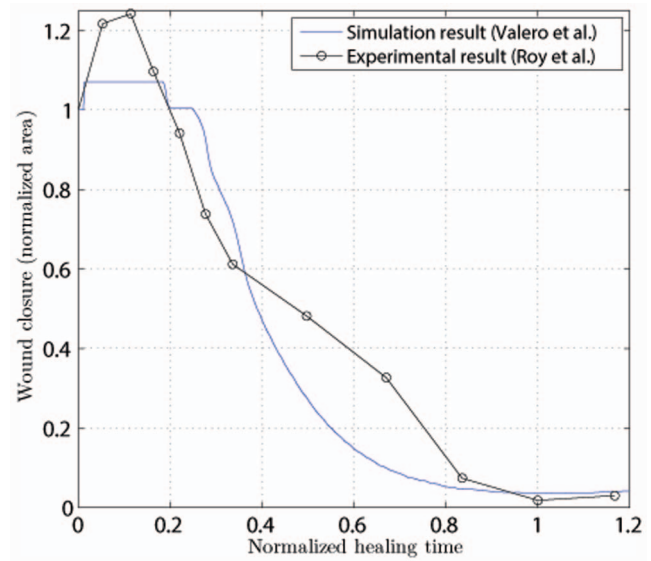


Figure 7. Normalized wound area as a function of the normalized healing time for a circular wound with a radius of 0.4 cm. Comparison with the experimental work of Roy et al.[34].
doi:10.1371/journal.pone.0092774.g007

where Q denotes the cellular/chemical species, \mathbf{J}_Q denotes its net flux over the domain of interest (which may include terms representing random dispersal -migration or diffusion-, directed migration (chemotaxis), and may also include a passive convection term due to ECM deformation), and f_Q denotes net production. The matrix deformation is obtained from the conservation of linear momentum

$$\nabla \cdot (\sigma_{ecm} + \sigma_{cell}) = \mathbf{f}_{subs} \tag{6}$$

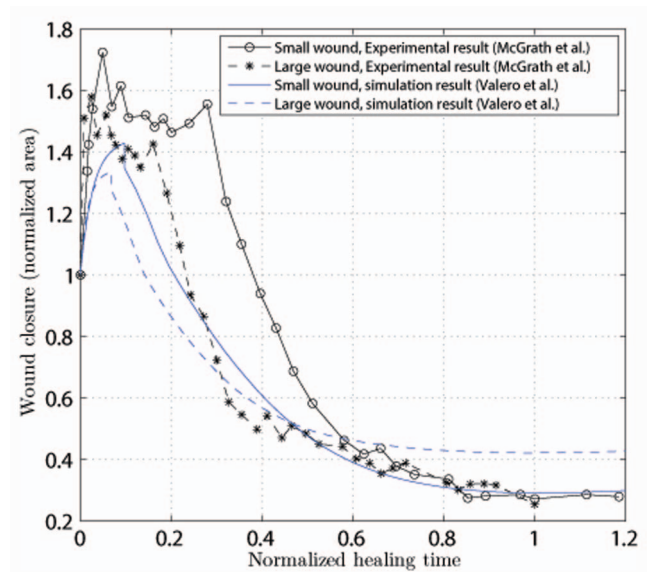


Figure 8. Normalized wound area as a function of the normalized healing time (time/healing time) for two square wounds with areas of 6.25 cm² and 12.54 cm². Solid lines refer to the small wound, and dashed lines refer to the large wound. Comparison with the experimental work of McGrath and Simon [35].
doi:10.1371/journal.pone.0092774.g008

Table 1. List of model parameters related to fibroblasts and myofibroblasts kinetics.

Parameter	Description	Value	Observations
n_0	fibroblasts density in undamaged dermis	10^4 cells/cm ³	[21]
D_n	fibroblasts diffusion rate	$2 \cdot 10^{-2}$ cm ² /day	[27] [†]
a_n	together with b_n determines the maximal chemotaxis rate per unit of GF concentration	$4 \cdot 10^{-10}$ g/cm day	[23]
b_n	GF concentration that produces 25% of the maximal chemotactic response	$2 \cdot 10^{-9}$ g/cm ³	[23]
r_n	fibroblasts proliferation rate	0.832 day ⁻¹	[27]
$r_{n,max}$	maximal rate of GF induced fibroblasts proliferation	0.3 day ⁻¹	[23]
$C_{1/2}$	half-maximal GF enhancement of fibroblasts proliferation	10^{-8} g/cm ³	[21]
K	fibroblasts maximal capacity in dermis	10^7 cells/cm ³	[21]
$k_{1,max}$	maximal rate of fibroblasts differentiation	0.8 day ⁻¹	[23]
C_k	half-maximal GF enhancement of fibroblasts differentiation	10^{-8} g/cm ³	[23]
k_2	myofibroblasts desdifferentiation rate	0.693 day ⁻¹	[23]
ϵ_r	proportionality factor	0.5	[21]

[†] Adjusted to fit reported migration rate with a traveling wave model.
doi:10.1371/journal.pone.0092774.t001

where σ_{ecm} denotes the passively resistant ECM stress, σ_{cell} denotes the ECM stress due to the cells-ECM adhesions and \mathbf{f}_{subs} denotes the ECM-substrate anchoring forces that resist ECM deformation.

This work follows the model proposed by Javierre et al. [23], based on the well-established model of Olsen et al. [21]. We consider the presence of two cellular species, fibroblasts (n) and myofibroblasts (m), embedded in a collagen (ρ) matrix and guided by the presence of a chemical growth factor (c). We also consider the matrix displacements (\mathbf{u}) as a primary variable in the model (see File S1).

Fibroblasts, connective tissue cells found in the skin, are the main cellular species involved in wound contraction. The main functions of fibroblasts are the synthesis of connective tissue in response to injury and remodeling of the collagen ECM by the exertion of traction forces [44]. Fibroblasts are motile cells that migrate by random dispersal, chemotaxis and passive convection caused by the ECM displacements. Hence, their net flux term can be written as

Table 2. List of model parameters related to collagen and growth factor kinetics.

Parameter	Description	Value	Observations
ρ_0	collagen concentration in undamaged dermis	0.1 g/cm ³	[21]
ρ_{mi}	initial collagen concentration in the wound	10^{-3} g/cm ³	[21]
c_0	GF concentration in the wound	10^{-8} g/cm ³	[21]
r_ρ	collagen production rate	$7.59 \cdot 10^{-10}$ g ³ /cm ⁶ cell day	$r_\rho = d_\rho \rho_0 (R_\rho^2 + \rho_0^2)$ [‡]
$r_{\rho,max}$	maximal rate of GF induced collagen production	$7.59 \cdot 10^{-9}$ g ³ /cm ⁶ cell day	[21]
C_ρ	half-maximal GF enhancement of collagen synthesis	10^{-9} g/cm ³	[21]
η	proportionality factor	2	[21]
R_ρ	half-maximal collagen enhancement of ECM deposition	0.3 g/cm ³	[21]
d_ρ	collagen degradation rate per unit of cell density	$7.59 \cdot 10^{-8}$ cm ³ /cell day	[21]
D_c	GF diffusion rate	$5 \cdot 10^{-2}$ cm ² /day	[21]
k_c	GF production rate per unit of cell density	$7.5 \cdot 10^{-6}$ cm ³ /cell day	[23] [§]
ζ	proportionality factor	1	[21]
Γ	half-maximal enhancement of net GF production	10^{-8} g/cm ³	[21]
d_c	GF decay rate	0.693 day ⁻¹	[23]

[‡] Determined collagen degradation kinetics to remain in equilibrium away from the wound.

[§] Downestimated to prevent fibro-proliferative disorders [22] with the used GF decay rates.
doi:10.1371/journal.pone.0092774.t002

Table 3. List of model parameters related to the mechanical behavior of cells and ECM.

Parameter	Description	Value	Observations
p_{max}	maximal cellular active stress per unit of ECM	10^{-5} N g/cm ² cell	[23]
K_{pas}	volumetric stiffness moduli of the passive components of the cell	$2 \cdot 10^{-5}$ N g/cm ² cell	[28]
K_{act}	volumetric stiffness moduli of the actin filaments of the cell	10^{-4} N g/cm ² cell	[28]
θ_1	shortening strain of the contractile element	-0.6	[23]
θ_2	lengthening strain of the contractile element	0.5	[28]
τ_d	half-maximal mechanical enhancement of fibroblast differentiation	10^{-5} N g/cm ² cell	[23]
μ_1	undamaged skin shear viscosity	200 N day/cm ²	[23]
μ_2	undamaged skin bulk viscosity	200 N day/cm ²	[23]
E	undamaged skin Young's modulus	3.34–33.4 N/cm ²	[51]
ν	undamaged skin Poisson's ratio	0.3	[51]
ξ	myofibroblasts enhancement of traction per unit of fibroblasts density	10- cm ³ /g	[21]
R_τ	traction inhibition collagen density	$5 \cdot 10^{-4}$ g/cm ³	[21]
s	dermis tethering factor	10^{-1} N/cm g	Estimated

doi:10.1371/journal.pone.0092774.t003

$$\mathbf{J}_n = -D_n \nabla n + \frac{a_n}{(b_n + c)^2} n \nabla c + n \frac{\partial \mathbf{u}}{\partial t}, \quad (7)$$

where D_n denotes the fibroblast diffusion rate, and a_n and b_n are chemotaxis-related parameters. The parameter values can be found in Table 1 and Table 2.

Fibroblasts kinetics is determined by their proliferation, differentiation into myofibroblasts, differentiation back from myofibroblasts and apoptosis. The novelty with respect to Javierre et al. [23] is the signal that triggers fibroblast differentiation. The ability of fibroblasts to sense the strain in the ECM [45] and the regulation of their differentiation to myofibroblasts by mechanical loads [30,31,46] are well known. Hence, we consider whether the differentiation process is driven by the deformation of the tissue where the cells are allocated instead of depending on the mechanical stress of the matrix itself [29]. We also maintain the hypothesis that this differentiation is also enhanced by different growth factors (e.g., PDGF and TGF- β) [31,47]. Hence, fibroblast differentiation into myofibroblasts can be expressed through the term

$$f_{diff} = -\frac{k_{1,max}c}{C_k + c} \theta^+ n. \quad (8)$$

where $k_{1,max}$ denotes the maximal rate of fibroblast differentiation and C_k regulates the influence of the growth factor during differentiation.

Fibroblasts differentiate into myofibroblasts under the influence of TGF- β and the resulting phenotype is able to exert and maintain higher contractile forces in the tissue [46]. Fibroblast differentiation into myofibroblasts occurs when the ECM has a positive volumetric strain, which is denoted by $\theta^+ = \max(\theta, 0)$. In this situation, cells are able to exert forces on the tissue, which means that the strain is not mainly caused by the tissue itself. Myofibroblasts are smooth muscle-like cells [31], which means that they are not motile and that their flux is only due to passive convection. Myofibroblast evolution is mainly due to proliferation,

differentiation from fibroblasts, inverse differentiation to fibroblasts and apoptosis [48].

Cells in the skin are embedded in the ECM, with the main components being collagen fibers produced by fibroblasts. Hence, we model the ECM density through the collagen density. Collagen fibers are non-motile, and hence their net flux term is expressed in terms of the passive convection of the skin.

Following the model of Olsen et al. [21] we consider the role of fibroblasts and myofibroblasts in collagen synthesis [31,49]. Furthermore, collagen production is enhanced by the presence of growth factors such as TGF- β [50].

The wound-healing process is regulated by several growth factors. Collagen-matrix contraction is regulated by PDGF [30] among other factors, and fibroblast differentiation is driven by TGF- β [31]. In this work, we consider a unique growth factor that regulates these processes for simplicity. The net flux of the growth factor is due to passive convection and also to diffusion through the tissue. Growth factor production is regulated by fibroblasts and myofibroblasts, following [23].

After a wound occurs, there is an instantaneous elastic response of the skin that causes the wound edge to retract, increasing the wound size. During this distraction process, the pre-stress of the skin is relaxed. Hence, the factors determining the change in wound geometry are purely mechanical. The time scale at which stress liberation occurs (on the order of minutes) is much smaller than the time scale at which cellular events such as migration, differentiation, proliferation and matrix production occur (on the order of days). Therefore, we assume that cells do not have time to influence the process, except by the death of cells due to the wounding process.

Once wound distraction has reached equilibrium, we consider the resulting wound geometry. The deformations accumulated until full wound distraction are also felt by the cells and they activate the mechanosensing mechanism controlling wound contraction. The skin is assumed to be a viscoelastic material [23,26].

The second major novelty of this work with respect to earlier works [21,23,25,26] rests on the expression for cell-induced stresses. The ECM deformation is obtained from the conservation of linear momentum (Equation (6)), where σ_{cell} denotes the cell-

exerted stresses. If we denote the traction force exerted by one fibroblast as $p_{cell}(\theta)$ [28], we can write the cell-induced stresses as

$$\sigma_{cell} = p_{cell}(\theta)(n + \xi m)I, \quad (9)$$

where the parameter values are included in Table 3.

Finally, we consider the observation that the ECM-substrate anchoring forces resisting ECM deformation are proportional to the tissue displacement and to the ECM density.

References

- Singer A, Clark R (1999) Mechanisms of disease - cutaneous wound healing. *New England Journal of Medicine* 341: 738–746.
- Li J, Chen J, Kirsner R (2007) Pathophysiology of acute wound healing. *Clinics in dermatology* 25: 9–18.
- Gurtner GC, Werner S, Barrandon Y, Longaker MT (2008) Wound repair and regeneration. *Nature* 453: 314–321.
- Vaughan M, Howard E, Tomasek J (2000) Transforming growth factor-beta 1 promotes the morphological and functional differentiation of the myofibroblast. *Experimental cell research* 257: 180–189.
- Wells RG (2008) The role of matrix stiffness in regulating cell behavior. *Hepatology* 47: 1394–1400.
- Discher D, Janmey P, Wang Y (2005) Tissue cells feel and respond to the stiffness of their substrate. *Science* 310: 1139–1143.
- Engler A, Griffin M, Sen S, Bonnetmann C, Sweeney H, et al. (2004) Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *Journal of Cell Biology* 166: 877–887.
- Harland B, Walcott S, Sun SX (2011) Adhesion dynamics and durotaxis in migrating cells. *Physical Biology* 8: 015011.
- Lo C, Wang H, Dembo M, Wang Y (2000) Cell movement is guided by the rigidity of the substrate. *Biophysical Journal* 79: 144–152.
- Saez A, Ghibaudo M, Buguin A, Silberzan P, Ladoux B (2007) Rigidity-driven growth and migration of epithelial cells on microstructured anisotropic substrates. *Proceedings of the National Academy of Sciences of the United States of America* 104: 8281–8286.
- Serra-Picamal X, Conte V, Vincent R, Anon E, Tambe DT, et al. (2012) Mechanical waves during tissue expansion. *Nature Physics* 8: 628–634.
- Vogel V, Sheetz M (2006) Local force and geometry sensing regulate cell functions. *Nature Reviews Molecular Cell Biology* 7: 265–275.
- Borau C, Kim T, Bidone T, Garcia-Aznar JM, Kamm RD (2012) Dynamic mechanisms of cell rigidity sensing: Insights from a computational model of actomyosin networks. *Plos One* 7: e49174.
- Jones C, Ehrlich HP (2011) Fibroblast expression of alpha-smooth muscle actin, alpha 2 beta 1 integrin and alpha v beta 3 integrin: Influence of surface rigidity. *Experimental and molecular pathology* 91: 394–399.
- Mitrossilis D, Fouchard J, Guirouy A, Desprat N, Rodriguez N, et al. (2009) Single-cell response to stiffness exhibits muscle-like behavior. *Proceedings of the National Academy of Sciences of the United States of America* 106: 18243–18248.
- Mitrossilis D, Fouchard J, Pereira D, Postic F, Richert A, et al. (2010) Real-time single-cell response to stiffness. *Proceedings of the National Academy of Sciences of the United States of America* 107: 16518–16523.
- Pelham R, Wang Y (1997) Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proceedings of the National Academy of Sciences of the United States of America* 94: 13661–13665.
- Adam J (2000) A simplified model of wound healing (with particular reference to the critical size defect) (vol 30, pg 23, 1999). *Mathematical and Computer Modelling* 31: 237–237.
- Murray JD, Cook J, Tyson R, Lubkin SR (1998) Spatial pattern formation in biology: I. dermal wound healing. ii. bacterial patterns. *Journal of the Franklin Institute-Engineering and Applied Mathematics* 335B: 303–332.
- Sherratt J, Murray J (1991) Mathematical-analysis of a basic model for epidermal wound-healing. *Journal of mathematical biology* 29: 389–404.
- Olsen L, Sherratt JA, Maini PK (1995) A mechanochemical model for adult dermal wound contraction and the permanence of the contracted tissue displacement profile. *Journal of theoretical biology* 177: 113–128.
- Olsen L, Sherratt J, Maini PK (1996) A mathematical model for fibroproliferative wound healing disorders. *Bulletin of mathematical biology* 58: 787–808.
- Javierre E, Moreo P, Doblare M, Garcia-Aznar JM (2009) Numerical modeling of a mechanochemical theory for wound contraction analysis. *International Journal of Solids and Structures* 46: 3597–3606.
- Murphy KE, Hall CL, McCue SW, McElwain DLS (2011) A two-compartment mechanochemical model of the roles of transforming growth factor beta and tissue tension in dermal wound healing. *Journal of theoretical biology* 272: 145–159.
- Murphy KE, Hall CL, Maini PK, McCue SW, McElwain DLS (2012) A fibrocontractive mechanochemical model of dermal wound closure incorporating realistic growth factor kinetics. *Bulletin of mathematical biology* 74: 1143–1170.
- Tranquillo R, Murray J (1992) Continuum model of fibroblast-driven wound contraction - inflammation-mediation. *Journal of theoretical biology* 158: 135.
- Ghosh K, Pan Z, Guan E, Ge S, Liu Y, et al. (2007) Cell adaptation to a physiologically relevant ecm mimic with different viscoelastic properties. *Biomaterials* 28: 671–679.
- Moreo P, Garcia-Aznar JM, Doblare M (2008) Modeling mechanosensing and its effect on the migration and proliferation of adherent cells rid f-8256-2010. *Acta Biomaterialia* 4: 613–621.
- Hinz B, Gabbiani G (2003) Mechanisms of force generation and transmission by myofibroblasts. *Current opinion in biotechnology* 14: 538–546.
- Grinnell F (2000) Fibroblast-collagen-matrix contraction: growth-factor signaling and mechanical loading. *Trends in cell biology* 10: 362–365.
- Tomasek J, Gabbiani G, Hinz B, Chaponnier C, Brown R (2002) Myofibroblasts and mechanoregulation of connective tissue remodelling. *Nature Reviews Molecular Cell Biology* 3: 349–363.
- Arora P, Narani N, McCulloch C (1999) The compliance of collagen gels regulates transforming growth factor-beta induction of alpha-smooth muscle actin in fibroblasts. *American Journal of Pathology* 154: 871–882.
- Hinz B, Mastrangelo D, Iselin C, Chaponnier C, Gabbiani G (2001) Mechanical tension controls granulation tissue contractile activity and myofibroblast differentiation. *American Journal of Pathology* 159: 1009–1020.
- Roy S, Biswas S, Khanna S, Gordillo G, Bergdall V, et al. (2009) Characterization of a preclinical model of chronic ischemic wound. *Physiological Genomics* 37: 211–224.
- McGrath M, Simon R (1983) Wound geometry and the kinetics of wound contraction. *Plastic and Reconstructive Surgery* 72: 66–72.
- Reina-Romo E, Gomez-Benito MJ, Garcia-Aznar JM, Dominguez J, Doblare M (2010) An interspecies computational study on limb lengthening. *Proceedings of the Institution of Mechanical Engineers Part H-Journal of Engineering in Medicine* 224: 1245–1256.
- Bidan CM, Kommareddy KP, Rumpel M, Kollmannsberger P, Brechet YJM, et al. (2012) How linear tension converts to curvature: Geometric control of bone tissue growth. *Plos One* 7: e36336.
- Radovanac K, Morgner J, Schulz JN, Blumbach K, Patterson C, et al. (2013) Stabilization of integrin-linked kinase by the hsp90-chip axis impacts cellular force generation, migration and the fibrotic response. *Embo Journal* 32: 1409–1424.
- Blustein D, Javaheri A (2008) Pressure ulcers: Prevention, evaluation, and management. *American Family Physician* 78: 1186–1194.
- Gaughitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG (2011) Hypertrophic scarring and keloids: Pathomechanisms and current and emerging treatment strategies. *Molecular Medicine* 17: 113–125.
- Aarabi S, Bhatt KA, Shi Y, Paterno J, Chang EI, et al. (2007) Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *Faseb Journal* 21: 3250–3261.
- Ogawa R (2011) Mechanobiology of scarring. *Wound Repair and Regeneration* 19: S2–S9.
- Guidry C (1992) Extracellular-matrix contraction by fibroblasts - peptide promoters and 2nd messengers. *Cancer and metastasis reviews* 11: 45–54.
- Grinnell F (1994) Fibroblasts, myofibroblasts, and wound contraction. *Journal of Cell Biology* 124: 401–404.
- Chiquet M, Reneda A, Huber F, Fluck M (2003) How do fibroblasts translate mechanical signals into changes in extracellular matrix production? *Matrix Biology* 22: 73–80.
- Grinnell F (2003) Fibroblast biology in three-dimensional collagen matrices. *Trends in cell biology* 13: 264–269.
- Grotendorst G, Seppa H, Kleinman H, Martin G (1981) Attachment of smooth-muscle cells to collagen and their migration toward platelet-derived growth-factor. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* 78: 3669–3672.

Supporting Information

File S1 Description of the Mechano-chemical Model and the Model Implementation.
(PDF)

Author Contributions

Conceived and designed the experiments: CV EJ JMGA MJGB. Performed the experiments: CV. Analyzed the data: CV EJ JMGA MJGB. Contributed reagents/materials/analysis tools: CV EJ JMGA MJGB. Wrote the paper: CV EJ JMGA MJGB.

48. Desmouliere A, Redard M, Darby I, Gabbiani G (1995) Apoptosis mediates the decrease in cellularity during the transition between granulation-tissue and scar. *American Journal of Pathology* 146: 56–66.
49. Serini G, Gabbiani G (1999) Mechanisms of myofibroblast activity and phenotypic modulation. *Experimental cell research* 250: 273–283.
50. Roberts A, Sporn M, Assoian R, Smith J, Roche N, et al. (1986) Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proceedings of the National Academy of Sciences of the United States of America* 83: 4167–4171.
51. Khatyr F, Imberdis C, Vescovo P, Varchon D, Lagarde JM (2004) Model of the viscoelastic behaviour of skin in vivo and study of anisotropy. *Skin Research and Technology* 10: 96–103.