Mathematical Modeling of Wound Healing and Subsequent Scarring

Literature Report

W. M. Boon

Nov 11$^{\text{th}}$, 2013

Chapter 1

Introduction

Wound healing is a process orchestrated by many closely related mediators. Each of these mediators plays its own specific role as the wound heals in a series of different stages. Using a mathematical model, we aim to simulate this phenomenon. We then expand on an existing model to include a more detailed version of the final stage in which contraction plays a role.

We will first explain what roles the different mediators have during the process of wound healing and how they affect each other. In order to mathematically model the entire event, some simplifications are made and the different influences are defined. We use the mathematical model presented in [3] in which we limit the mediators to two cell-types, two types of cytokines and growth factors, and two types of fiber species. These mediators are discussed in detail with an emphasis on the mathematical implementation. A few improvements are then discussed as well as the plan for further research.

This further research will mostly consist of an expansion of the existing model in which contraction of the scar tissue is simulated. Contraction occurs in the final stage of healing when cells of a third type start pulling on the surrounding tissue [1]. This causes the area of the wound to decrease and ultimately speed up the healing process. In wounds with a larger area however, this contraction can lead to serious consequences. Due the tightness and decreases elasticity of the skin, the patient could suffer from a loss in mobility.

A second interesting result we plan to simulate is the orientation of the fibers in the extracellular matrix of skin. Uninjured skin has its fibers aligned isotropically which results in strength against forces from all directions. In scar tissue, however, the fibers seem to be aligned anisotropically [2]. This means that the scar tissue created after healing turns out to be inferior in strength to healthy skin tissue.

Chapter 2

Biological Processes

In this report, we will focus on the main stages called inflammation, proliferation and remodeling. Even though these stages overlap slightly, they are well-defined and form the entire process from the initiation of the wound up to the final scar tissue [1]. We will now give a short description of each of these stages.

2.1 Inflammation

The inflammatory stage takes place in the first three days of wound healing. In this phase, the wound is filled with blood and a fibrin-rich clot is formed. Due to the production of tissue plasminogen activator (tPA) at the edges of the wound, this fibrin clot is slowly broken down so that it can be replaced by granulation tissue. Granulation tissue consists of blood vessels, macrophages, fibroblasts and loose connective tissue [3]. The first cells to arrive are macrophages, which are attracted by the elevated concentrations of cytokines in the wound. The macrophages which migrate into the wound will then produce transforming growth factor-β (TGF-β) [1].

2.2 Proliferation

The higher concentration of TGF-β will serve as an attractant of the second type of cells, namely fibroblasts. This migration will start the second stage of wound healing called the proliferation stage which starts about three days after wounding. As more fibroblasts migrate into the wound and start proliferating, they start creating collagen fibrils. These fibrils will form the building blocks of the new extracellular matrix. During this stage, the granulation tissue will also be fully established [1].

2.3 Remodeling

The final stage consists mostly of the continued interaction between fibroblasts and collagen. The extracellular matrix is constantly remodeled as fibroblasts synthesize and break down collagen fibrils until approximately 3 weeks after wounding. Because of this interaction, fibroblasts cause contraction of the wound which makes the wound area smaller. This happens in order to speed up the healing process [1] so that hazardous external chemicls cannot easily invade the tissue. In fact, this is an evolutionary survival mechanism.

Chapter 3

Mathematical Implementation

3.1 Domain

The domain consists of a cross-section of the upper part of the skin. Here, an incision is made in the top region which will act as the wound space surrounded by healthy tissue as shown in Figure 3.1. The size of this domain is 1200 μm in width and 1600 μm in height. The domain is scaled by a factor of 400 μm in each direction resulting in a domain of 3×4. The same is done for the wound space which was originally $400\mu\text{m} \times 800\mu\text{m}$ and therefore becomes 1×2.

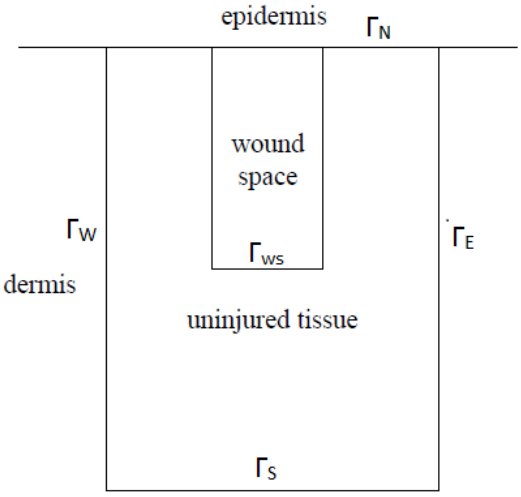

Figure 3.1: The domain of computation showing the wound space at the top surrounded by healthy tissue and the four borders. The boundary between the wound space and healthy tissue will be denoted by Γ_{ws}.

3.2 Main Factors

In order to construct the mathematical model, we need to define the main mediators. These mediators can be classified into three types: cells, cytokines and fibers. The cells will be implemented as discrete entities moving throughout the domain whereas the other factors will be defined on a triangular mesh. A finite element method is then used to solve the corresponding partial differential equations.

The different mediators have several influences on each other as visualized in Figure 3.2.

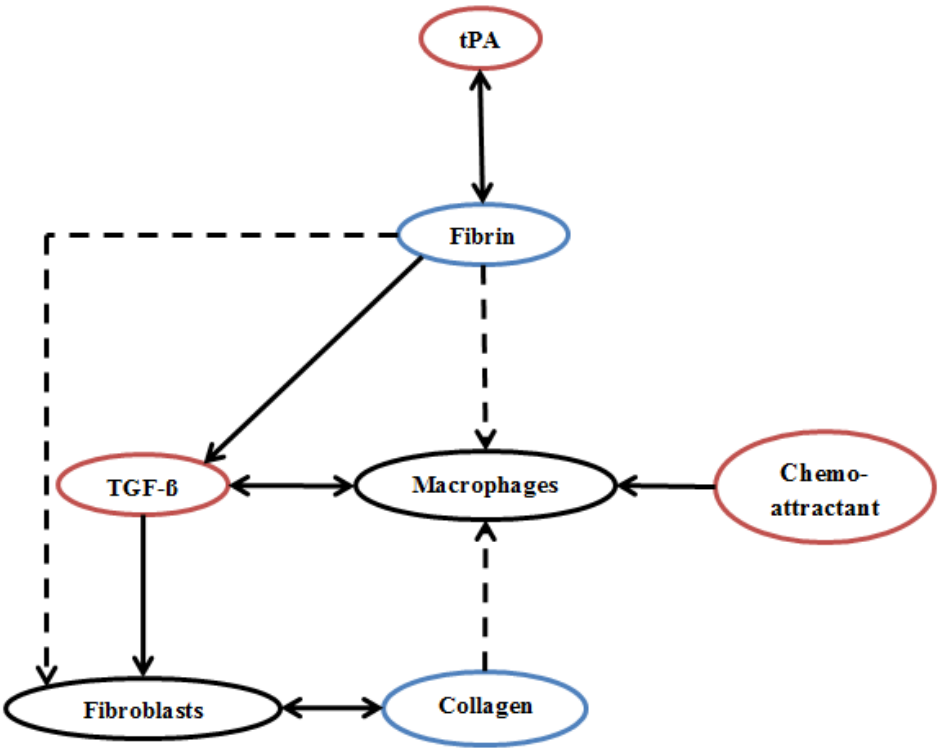

Figure 3.2: A simplified representation of the direct influences of the main mediators on each other. Dashed lines represent an influence on velocity.

3.2.1 Cytokines

The two types of cytokine used in [3] are tPA (ϕ_p) and TGF-β (ϕ_t). Both of these cytokines are modeled using a diffusion equation with a source term:

$$\frac{\partial \phi_k}{\partial t} = \nabla \cdot (D_k(\rho_f) \cdot \nabla \phi_k) + T_k, \quad k \in \{p, t\}. \tag{3.1}$$

The diffusion term depends on the density of the fibrin clot (ρ_f) which is assumed to decrease linarly as the density increases from zero to unity. The diffusion is defined as follows:

$$D_k(\rho_f) = D_k^{max}(1 - \rho_f) + \rho_f D_k^{min}, \quad k \in \{p, t\}.$$

A list of the used constants is given in the appendix.

The source term T differs for tPA and TGF-β. In the case of tPA, the source is high along the borders of the wound space and zero in the rest of the domain. In [3], this is modeled using a discontinuous source function. However, this will decrease the accuracy in our finite element framework. Furthermore, it is unrealistic to assume that the cytokine is only produced in such a clearly marked area. Instead of the given source function, we have chosen to model it as a hyperbolic tangent depending on the distance to the wound edges (Γ_{ws}). With a few chosen constants to fit the requirements, the source term becomes the following which is plotted in Figure 3.3:

$$T_p(x) = \frac{1}{20} \cdot \left[\frac{1}{2} + \frac{1}{2} \tanh\left(\frac{0.05 - \|x - \Gamma_w\|}{0.1} \right) \right].$$

In the case of TGF-β, the sources are the macrophages. Since these cells are relatively small, we will model them as point sources using Dirac delta functions. Furthermore, the production of TGF-β

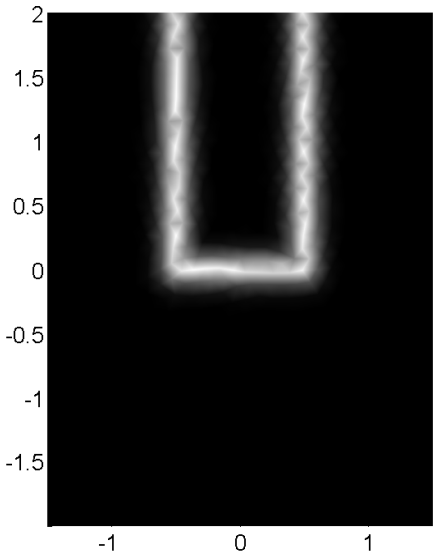

Figure 3.3: The source function of tPA showing the production along the edges of the wound space.

decreases when a macrophage has a higher proportion of bound receptors (n_i) and increases when there is contact with more fibrin (ρ_{f_i}). With the use of the right values, the source term becomes:

$$T_t(x,t) = 3.13 \cdot 10^{-5} \cdot \sum_{i=1}^{N_{macro}} (1 - 0.25 n_i) \cdot (0.10 + 0.90 \rho_{f_i}) \cdot \delta(x - x_i^m(t)).$$

Before finalizing the cytokine implementation, we need to look closely at the interaction between macrophages and TGF-β. In [2], macrophages produce TGF-β and move in the direction of the gradient of TGF-β as well. As a result, the macrophages start to cluster outside of the wound space towards the peak in TGF-β which they have created themselves. Since this is incorrect, their migration should not be modeled using the gradient of TGF-β but with an added cytokine PDGF (ϕ_c). The PDGF concentration is simply modeled using equation 3.1 without the source term and an initial condition such that the macrophages migrate into the wound. Again, this is accomplished with the use of hyperbolic tangents and is shown in Figure 3.4.

The boundary conditions for all cytokine equations are the same. Since the north boundary Γ_N represents the surface of the skin, a no-flux condition is imposed on this boundary:

$$\left.\frac{\partial \phi_k}{\partial \underline{n}}\right|_{\Gamma_N} = 0, \quad k \in \{p, t, c\}$$

where $\underline{n}$ is the normal vector pointing out from the boundary. On the other boundaries, the cytokines should be able to diffuse towards the outside of the domain. Since we assume that these cytokines are not present there, a Robin condition is imposed:

$$\left.\frac{\partial \phi_k}{\partial \underline{n}}\right|_{\Gamma_E \cup \Gamma_S \cup \Gamma_W} = -\phi_k, \quad k \in \{p, t, c\}.$$

3.2.2 Fibers

The two species of fiber are fibrin (ρ_f) and collagen (ρ_c). The main difference in their implementation is that the orientation of fibrin is not important hence we only incorporate the fibrin density in the model. For collagen, both the density and the orientation are saved in an orientation tensor (Ω) as

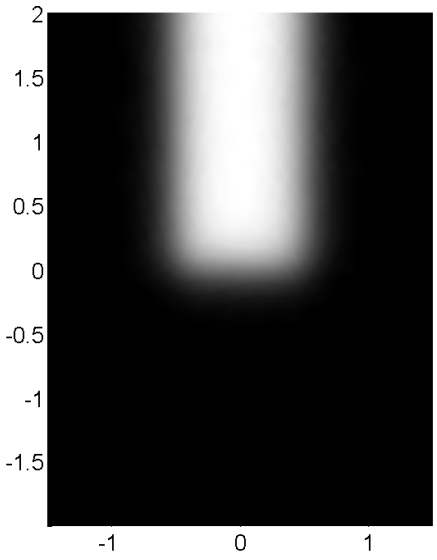

Figure 3.4: The initial distribution of the added cytokine which has a high concentration in the wound space.

described by Cumming in [3]. The eigenvectors of the tensor with their corresponding eigenvalues will then represent the orientation of the fibrils.

Fibrin

The only factor affecting the fibrin density is the concentration of tPA which causes it to degrade. With the use of a degradation factor r_p, we create the following formula:

$$\frac{\partial \rho_f}{\partial t} = -r_p \phi_p \rho_f.$$

In the beginning, the wound space is filled with the fibrin clot. Therefore, the initial condition for the fibrin concentration is one inside the wound and zero outside of it. Again, this discontinuous assumption is not realistic and we replace this with a function created by hyperbolic tangents.

Collagen

For the implementation of the collagen, its orientation is just as important as the density ρ_c. Therefore, Cumming's work describes a tensor approach for the orientation of the collagen fibers in [2] which will be denoted by Ω. The collagen density is saved in the trace of the tensor

$$\mathrm{tr}(\Omega) = \rho_c,$$

and the orientation of the fibers is defined by the eigenvectors with their corresponding eigenvalues. With this approach, we can easily calculate a metric for isotropy within the clot by comparing the magnitude of the eigenvalues.

The tensor representation satisfies the following differential equation:

$$\frac{\partial \Omega}{\partial t} = (1 - \rho_f - \rho_c) \cdot \sum_{i=1}^{N_{fibro}} (k_1 + (k_2 - k_1) n_i) \cdot \underline{\hat{g}}_i \underline{\hat{g}}_i^T \cdot \delta(x - x_i).$$

The first factor ensures that the sum of the fibrin and collagen density does not exceed unity. A sum is then made over all fibroblasts; the cells responsible for collagen production. They will reorient the collagen according to their own (normalized) movement direction $\hat{\underline{g}}_i$. The amount of collagen produced depends on how many bound TGF-β receptors the fibroblast has with k_1 as a lower limit and k_2 as the upper limit. Again, the use of Dirac-delta functions enables us to consider the fibroblasts as point sources.

The initial condition for the collagen concentration is that the uninjured tissue consists of isotropically oriented collagen and there is no collagen present in the wound space. The isotropy will be implemented by initially setting each orientation tensor as a factor times the identity matrix. Since the collagen density equals the trace of this tensor, this factor will be half of the density.

Once again, the discontinuity in the initial condition is improved using hyperbolic tangents.

3.2.3 Cells

As explained earlier, there are two types of cells in this model, namely macrophages and fibroblasts which both react to the concentration of TGF-β in their environment. However, since the cells do not respond immediately, the model introduces TGF-β receptors so that these first have to be bound to TGF-β before a reaction will occur. The proportion of bound TGF-β receptors (n) will act according to the following differential equation:

$$\frac{\partial n_i}{\partial t} = -\beta_t n_i + \gamma_t \phi_t (1 - n_i).$$

Here, β_t is the factor for the natural unbinding of the receptors while γ_t is the factor for receptors binding to TGF-β. The term $(1 - n_i)$ ensures that the proportion of bound receptors does not exceed one.

Another similarity between the cells is their movement speed. As described in [3], cell movement is split up into a normalized direction vector and the velocity size. The speed for both cell types is defined as follows:

$$v_i = v_{max}(1 - k_6\rho_f) \cdot (1 - k_7\rho_c) \cdot \left(\frac{1}{4} + \frac{3}{4 + 80(1 - n_i)^6}\right).$$

As this equation shows, the movement speed declines linearly as the density of fibrin or collagen increases. Furthermore, a higher proportion of bound TGF-β receptors will result in a higher speed. This last dependency on bound receptors is shown in Figure 3.5.

Macrophages

For the second part of the cell movement, a direction needs to be given. The macrophages will move in the direction of the added PDGF (ϕ_c) as discussed in chapter 3.2.1. However, this response is not instantaneous and therefore the direction $\mathbf{g}_m$ in which a macrophage moves is defined by a differential equation:

$$\mathrm{d}\mathbf{g}_m = (-\beta \mathbf{g}_m + \gamma(1 - n_i)\nabla\phi_c)\mathrm{d}t + \sqrt{\alpha}\mathrm{d}\mathbf{W}. \tag{3.2}$$

Again we introduce two constants. First, β defines the directional persistence of the cells restricting cells from instant changes in direction. Then, γ is the sensitivity factor of the macrophages movement to the gradient of the chemoattractant ϕ_c. The term $(1 - n_i)$ is responsible for the influence of bound TGF-β receptors. The last term in this equation describes a random deviation in the form of a random walk with scaling factor $\sqrt{\alpha}$.

Since these cells are described as discrete individuals, the direction needs to be changed once a collision happens. As shown in Figure 3.6, a new direction can be chosen so that the deviation from its original path is minimal. This direction can always be chosen except in the special case where the angle θ equals $\pm\frac{1}{2}\pi$ which means that the cell has a 'head-on' collision and is forced to stop.

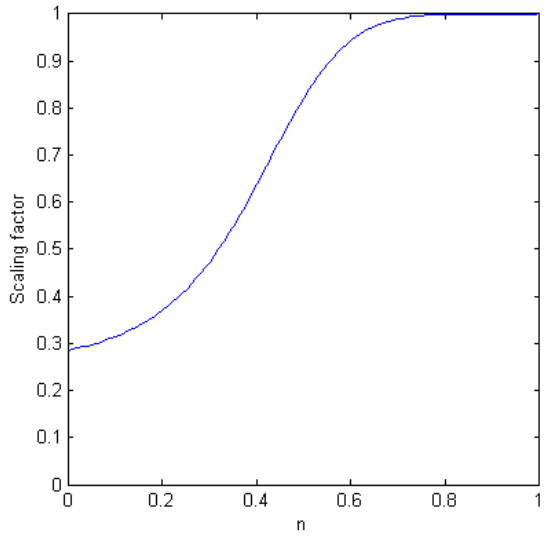

Figure 3.5: Cell speed scaling factor depending on proportion of bound TGF-β receptors.

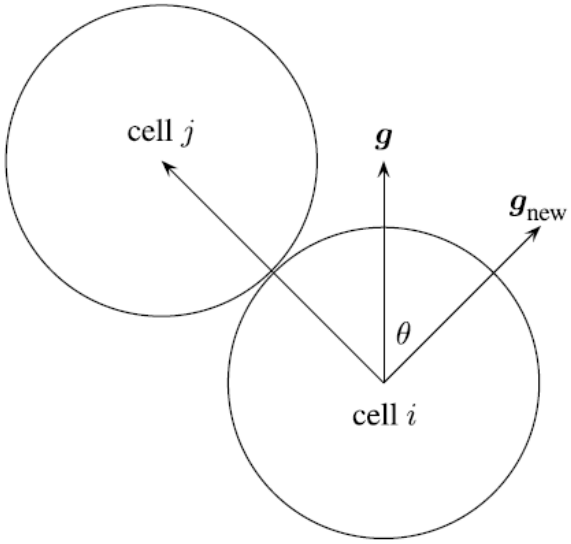

Figure 3.6: When two cells collide, the direction is changed with a minimal deviation.

Fibroblasts

The differential equation for the direction of fibroblasts $\mathbf{g}_f$ is the same as for the macrophages in 3.2 except for one difference. Since fibroblasts move towards higher concentrations of TGF-β, the term $\nabla\phi_c$ is replaced with $\nabla\phi_t$. Furthermore, fibroblasts are subject to contact guidance of the collagen fibers. The direction in which it moves is therefore altered according to the following equation:

$$\mathbf{g} = ((1-\rho_c)\mathbf{I} + \rho_c\hat{\mathbf{\Omega}})\mathbf{g}_f.$$

Here, $\hat{\mathbf{\Omega}}$ is the normalized (length-preserving) orientation tensor of collagen at the location of the fibroblast and $\mathbf{I}$ is the 2×2 identity matrix. If the collagen density is zero, there is no contact guidance while a higher collagen density results in a higher influence on the fibroblast's direction.

Just like the macrophages, fibroblasts are subject to collisions. The same technique is used to find a direction with the smallest deviation from its path in case of a collision.

Chapter 4

Further work

First of all, some improvements need to be made for the model. Let us consider the technique used to describe cell collisions. Even though this technique seems to work well with small time steps, it fails when the time steps get bigger. To improve this part of the model, we will use [4] in which contact forces are formulated. These forces are made to push cells away from each other once they get too close. Using this approach, a much more realistic view can be made showing cells pushing each other out of the way. This can simply not be achieved with the formulation of Cumming in [3].

Furthermore, we have already shown that a third cytokine is required to attract the macrophages into the wound space. This has to be done so that the macrophages do not cluster in the areas where they have created the TGF-β themselves.

The model by Cumming is based on incision-like wounds. It is assumed that the area of the wound is so small that contraction of the wound space may be neglected. In order to improve this model for wounds with a larger area, it is interesting to look at the effects of contraction during the remodeling stages.

The process of contraction occurs when fibroblasts differentiate to become myofibroblasts which can be seen as a cell in between a fibroblast and a smooth muscle cell. These cells pull on the surrounding tissue causing the wound space to contract. In small wounds this will speed up the healing process but it may cause major problems in wounds with a larger area. Scar tissue is less elastic than healthy skin after contraction, thus this deformation could cause a loss of mobility for the patient.

To implement this, we must change the domain so that we no longer consider a cross-section of the skin but instead look at a wound from above. This way, we can study what happens to the boundaries of the wound space as it undergoes the process of contraction.

In the end it will be interesting to compare the results from the finite element method to a more analytical approach using Green's functions. We will investigate whether the same results can be obtained with a method which is computationally much cheaper.

Appendix A

List of constants

Constant	Value	Description
D_p^{min}	$3.13 \cdot 10^{-4}$	Minimum diffusion tPA.
D_p^{max}	$6.25 \cdot 10^{-3}$	Maximum diffusion tPA.
D_t^{min}	$4.69 \cdot 10^{-4}$	Minimum diffusion TGF-β.
D_t^{max}	$4.69 \cdot 10^{-3}$	Maximum diffusion TGF-β.
k_1	$1.88 \cdot 10^{-4}$	Minimum collagen synthesis.
k_2	$3.75 \cdot 10^{-4}$	Maximum collagen synthesis.
k_6	0.40	Influence of fibrin density on cell movement.
k_7	0.60	Influence of collagen density on cell movement.
r_p	0.15	Factor of fibrin decay.
β_t	1	Rate of unbinding TGF-β receptors.
γ_t	5	Rate of unbinding TGF-β receptors.
v_{max}^m	1	Maximum velocity for macrophages.
v_{max}^f	0.38	Maximum velocity for fibroblasts.
β_m	2	Factor of directional persistence macrophages.
γ_m	5	Directional sensitivity to gradient of chemoattractant for macrophages.
β_f	1	Factor of directional persistence fibroblasts.
γ_f	10	Directional sensitivity to gradient of chemoattractant for fibroblasts.
α	10^{-4}	Standard deviation in the random walk.

Table A.1: The values of the constants used in this report.

Bibliography

[1] Enoch, S., Leaper, D.J.: *Basic science of wound healing*, (2008)

[2] Cumming, B.D.: *A Mathematical Model of Wound Healing and Subsequent Scarring*, (2006)

[3] Cumming, B.D., McElwain, D.L.S., Upton, Z.: *A mathematical model of wound healing and subsequent scarring*, J.R. Soc. Interface(2010)

[4] Vermolen, F.J., Gefen, A.: *A semi-stochastic cell-based formalism to model the dynamics of migration of cells in colonies*