



Modeling the effects of transforming growth factor- β on extracellular matrix alignment in dermal wound repair

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We present a novel mathematical model for collagen deposition and alignment during dermal wound healing, focusing on the regulatory effects of transforming growth factor- β (TGF β .) Our work extends a previously developed model which considers the interactions between fibroblasts and an extracellular matrix composed of collagen and a fibrin based blood clot, by allowing fibroblasts to orient the collagen matrix, and produce and degrade the extracellular matrix, while the matrix directs the fibroblasts and control their speed. Here we extend the model by allowing a time varying concentration of TGF β to alter the properties of the fibroblasts. Thus we are able to simulate experiments which alter the TGF β profile. Within this model framework we find that most of the known effects of TGF β , i.e., changes in cell motility, cell proliferation and collagen production, are of minor importance to matrix alignment and cannot explain the anti-scarring properties of TGF β . However, we find that by changing fibroblast reorientation rates, consistent with experimental evidence, the alignment of the regenerated tissue can be significantly altered. These data provide an explanation for the experimentally observed influence of TGF β on scarring. (WOUND REP REG 2001;9:278–286)

Typically wound repair results in a scar where the newly formed tissue is distinctly different from the old (normal) tissue, and less functional. Recent studies show that altering the profile of transforming growth factor- β (TGF β) during healing can have profound effects on the healing process, including significantly increasing or decreasing the degree of scarring.^{1–4} It is known that TGF β alters the wound healing process in many ways,^{5,6} and it is unclear which of these effects are responsible for changing the degree of scarring. The basic objective of this study was to use mathematical modeling to determine those aspects of TGF β activity that were most significant for wound repair.

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TGF β Transforming growth factor- β

One of the main differences between scar tissue and normal tissue is the alignment of the extracellular matrix. For example, in normal rodent tissue the collagen fibers of the extracellular matrix are randomly aligned, whereas in scar tissue they are primarily aligned perpendicular to the basement membrane.⁷ Other differences include higher density of collagen, thicker fibers, and lower tensile strength in scar tissue, as well as different ratios of the types of collagen present.⁸ In this article we are primarily concerned with the difference in alignment, which is currently thought to play a major role in determining scar quality.⁹

The model simulates the invasion of the wound region by surrounding fibroblasts, which are guided by the extracellular matrix. The cells in turn modify the extracellular matrix, which for simplicity we assume to be composed only of fibrin and collagen, by degrading, producing and aligning the proteins. By using this mathematical model, which focuses on simple interactions be-

tween the fibroblasts and the extracellular matrix, we can easily manipulate the system in a manner unavailable in experiments, and can thus help to understand the complex dynamics of wound healing. Within this modeling framework, we can explore the consequences of TGF β -regulation of fibroblast properties.

MATERIALS AND METHODS

Our model is an extension of one presented previously,¹⁰ which considers three components of a wound: fibroblasts, collagen and fibrin. The new ingredient here is that we include explicitly the dependence of fibroblast properties on TGF β . The collagen and fibrin networks are described by continuous functions representing direction (the predominant local fiber direction) and density, and the fibroblasts move and modify these networks. We separately track the movement of each fibroblast cell. The concentration of TGF β is given by a function which varies with time but is constant throughout the wound region, which is taken to be a two-dimensional cross section of a slash wound. Typically it is 1 millimeter deep and 0.5 millimeters wide.

Within this framework we incorporate a range of cell–extracellular matrix interactions. The fibroblasts are guided by the extracellular matrix, which is composed of collagen and fibrin. This well-documented phenomenon is known as contact guidance.^{11–13} In our model, we assume that as the cells move, they sample their environment, the extracellular matrix, and determine which direction to move in, based on the local fiber orientation (see the appendix for details). Additionally, their speed is affected by the density of the different proteins. In particular, the fibroblast speed depends on fibronectin in a biphasic manner,¹⁴ with cells moving more slowly at low or high density. We are assuming that the fibronectin concentration is proportional to the fibrin concentration and that the densities are limited to the range where the speed increases. Thus we take the cell speed to increase linearly with fibrin concentration. The response to collagen density is similarly biphasic, but the cell speed is more sensitive to fibronectin¹⁵ than to collagen. Specifically, we assume that the extracellular matrix can alter the cell's speed by a factor of about 8, with the average speed being between 2 to 5 microns per hour. The reader is referred to the appendix and previous work¹⁰ for the precise functional forms and more details.

The cells are modeled to allow polarization, that is, their direction of motion is influenced by their previous direction. The cells enter the wound region from the surrounding tissue and proliferate by cell division. Exper-

iments suggest that most of the fibroblasts in the wound are derived from the subcutaneous fascia¹⁶ and in most simulations we assume that 80 percent of the cells entering the wound from surrounding tissue come from the fascia. The total number of cells in the wound region varies with time. Thus the proportion that migrate from the surrounding tissue and the proportion of cells due to cell proliferation depend on time and the proliferation rate.

Fibroblast behavior is determined by the extracellular matrix, but the cells also modify the extracellular matrix around them. Thus in our model the cells degrade collagen and fibrin as well as produce collagen. The simulated cells degrade collagen (and fibrin) at a rate proportional to the amount of collagen (or fibrin) already present, and produce collagen at a constant rate, oriented in the direction of movement. This allows us to simulate the transformation, via fibroblasts, of a fibrin blood clot into an extracellular matrix composed primarily of collagen. As the cells change the composition of the extracellular matrix, they also align the matrix.^{17,18} It is unclear whether this alignment is due only to mechanical forces the cells exert on the matrix as they move,¹⁹ or other more local influences due to the production of new fibers. In our model we do not distinguish between these different effects: we simply assume alignment is a local effect, in which the fibroblasts align the collagen matrix (but not fibrin) towards the direction in which they are moving.

There are many sources of TGF β in the wound, including monocytes, fibroblasts, and platelets, which seem to be the earliest source.⁵ In view of this range of sources, and because the wound region is relatively small with no experimental data to quantify spatial variations, we assume a uniform concentration of TGF β in the wound region which varies with time.

It is known that TGF β has various effects on fibroblasts: it acts as a chemoattractant, modulates collagen production and degradation as well as cell proliferation.^{20,21} In our mathematical model it is easy to incorporate all of these effects, but more importantly we can separate them, and study how they relate to one another. In the remainder of this paper we give results of simulations which examine the individual effects of TGF β , the effects of different isoforms of TGF β , and finally we end with a discussion of our findings. But we begin by giving further details about the model.

RESULTS

Our study of TGF β activity was made possible by the recent data of Yang and coworkers,²² who determined a detailed time-course of active TGF β in dermal wound

healing in rats. They found that the concentration of active TGF β peaks within an hour after wounding, decreases to a level above normal within one day, peaks again five days after wounding and then more gradually decreases until reaching approximately normal levels 14 days after wounding. Because fibroblasts do not enter the wound until 24 to 48 hours after wounding, we ignore the first peak of TGF β concentration and use the functional form shown in Figure 1 for the TGF β concentration.

We begin by considering separately the effects of TGF β on cell proliferation, cell motility, and collagen production.

Effect of TGF β on cell proliferation

The effect of TGF β on cell proliferation depends on the developmental age of the source of the fibroblasts, with fibroblasts from younger sources having a greater proliferative response to the growth factor ranging from just over 100 percent of normal to over 200 percent of normal.^{23,24} The response depends on growth factor concentration in a biphasic manner, increasing and then decreasing with concentration, with the maximum response of over 250 percent above normal at a concentration of 1 pM in monolayers.²⁰ We allow the fibroblast proliferation to vary with TGF β concentration in a linear manner, increasing the proliferation up to a maximum of two times that of normal. This results in a change from 66 percent of the total number of cells at the end of the simulation resulting from cell proliferation to 77 percent. Previous investigations¹⁰ showed that greatly increased cell division tended to randomize the fiber alignment due to a decrease in the number of cells entering from the surrounding tissue (the maximum cell den-

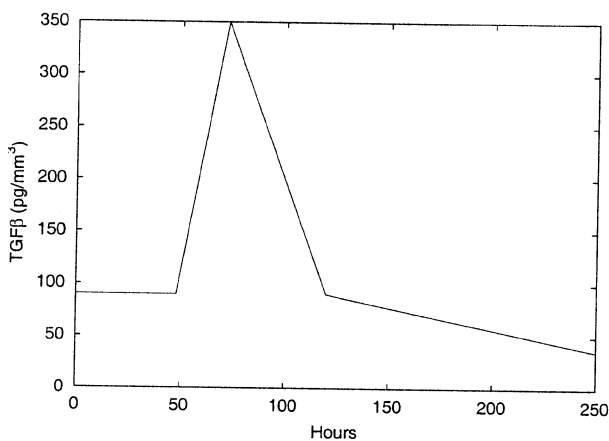


Figure 1. A graph of the function used for TGF β concentration. Time $t = 0$ corresponds to 48 hours after wounding, which is when fibroblasts begin entering the wound. This graph is based on data taken from Yang et al.²²

sity is kept constant), and due to our assumption that cells lose their polarization when they divide. However, our simulations show that the relatively slight increases (a factor of 2 at most) in cell proliferation caused by TGF β has little effect on the fiber alignment. The density distribution of the wound region changes, with more dense collagen in the center towards the base of the wound because the cells rapidly accumulate in the wound region when there is increased proliferation, the overall collagen density is typically higher at any given time post-wounding.

The response to TGF β is biphasic and proliferation is less at the highest concentrations reported. These concentrations are of the same order of magnitude as the concentration reported in the wound, so we must consider the possibility that proliferation decreases as the TGF β concentration peaks. Thus, in contrast to our previous simulation, we begin with a proliferation rate 2 times higher than normal, decreasing with increasing TGF β concentration. This is consistent with the experimentally measured cell proliferation in the wound region, which falls dramatically between day 5 and day 7 post-wounding.¹⁶ Surprisingly, this results in more alignment of the collagen fibers (Figure 2b). In this simulation the cells do not get slowed down in high density regions near the boundary of the wound, and thus are more uniformly distributed throughout the wound region, leading to increased alignment.

Effect of TGF β on cell motility

As in the case of cell proliferation, the experimental evidence does not indicate a simple relationship between fibroblast motility and TGF β levels. Fibroblasts show a biphasic response to TGF β concentrations, and the maximum response involves 400 percent more cells migrating than normal.²⁰ Furthermore, it was reported that this increased migration was not due to chemokinesis but was a chemotactic response: that is, there is not an increase in overall motility, but a directed movement along chemical gradients. In other work where fibroblasts are placed on 3-dimensional collagen gels and their motility is studied by examining how many invade the gel, the effect of TGF β again depends on the developmental age of the source of fibroblasts as well as the cell density.^{25,26} It was found that at low densities half as many adult fibroblasts migrated into the gel with increased TGF β concentration. In this study TGF β was uniformly increased so there were no chemical gradients. The results from both of these studies suggest that TGF β causes a chemotactic response and alters the speed of the cells. In our model we were able to alter those two components: we linearly decreased the cell speed up to



Figure 2. The collagen matrix is represented for four different simulations. The individual effects of TGF β are shown starting with (a) no effects of TGF β , (b) cell proliferation, (c) cell migration, and (d) collagen production. In (a) the simulation corresponds to treatment with an antibody for TGF β . In (b) the cell proliferation starts out at twice the rate used in (a) and linearly decreases to one as TGF β concentration increases. In (c) the cell speed decreases up to a factor of one half and the cell flux decreases from four times the rate used in (a) to one times the rate as TGF β concentration increases. Finally, in (d) the collagen production is allowed to increase to three times the rate used in (a) as TGF β concentration increases. The region represents a cross section of a slash wound with the surface of the skin at the top, normal tissue to the right and left and the basement membrane at the bottom. The results are shown after 240 hours of simulation corresponding to about 12 days after wounding (the fibroblast enter the wound region between 24–48 hours after wounding). Black indicates high density and white indicates no collagen. The line segments are used to represent the collagen fibers and their orientation. In reality they are curves whose tangent lines correspond to the direction of the vector field used to represent the collagen or streamlines.

a factor of one half depending on the TGF β concentration, and we increased the cell flux into the wound region by up to a factor of four, again depending on the TGF β concentration. Cell speed can have significant effects on collagen alignment, because in the model, cells move in one direction for a fixed time interval, before (potentially) reorienting. Thus when the cells move faster, they move a greater distance in a particular direction during this fixed time interval, causing more alignment.¹⁰ In this case the speed decrease is relatively slight, and causes the collagen to be only slightly less aligned. In contrast, the increased flux significantly increases alignment perpendicular to the skin surface, since 80 percent of the cells are assumed to enter the wound from underlying tissue, and only 20 percent from the surrounding dermis.¹⁶ Combining both effects gives a net increase in alignment, because the alignment due to the flux overcomes the randomizing influence of the decreased speed. Because the chemotactic response is biphasic, we also ran a simulation where the peak in TGF β concentration decreased the cell flux from the elevated levels. That

simulation gave results that were similar to the increasing flux case, but with slightly more alignment (Figure 2c).

Effect of TGF β on collagen production

Because the speed of the fibroblasts depends on the collagen density, changes in collagen production rate affect the speed of the cells and therefore the alignment of the collagen fibers. By increasing collagen production²⁷ and decreasing collagenase production,²⁸ TGF β modifies the overall extracellular collagen density.²⁹ At TGF β concentrations comparable to those found in wounds, the overall collagen accumulation is increased by a factor of two.³⁰ At lower concentrations, the increase in collagen was found to range from two- to three-fold and was dependent on the amount of collagen already present in the medium.²¹ In our model simulations we allow the collagen production to increase linearly to a maximum of three times normal production. This results in a less aligned collagen gel since, as the overall collagen density is higher, the cells are slightly slower and the reduced speed decreases the alignment (Figure 2d).

Taken together, these results show that the effects of TGF β on cell proliferation, movement and collagen production are unable to explain the experimentally observed collagen alignment patterns. This is illustrated in Figure 3a, c, which shows the combined effect of these three regulatory functions of TGF β . Moreover, corresponding computer simulations in which the TGF β concentration is set to zero (Figure 3b, d) show a net increase

in alignment, in contrast to the observed reduction in scarring in wounds treated with antibodies for TGF β . This indicates that other regulatory functions of TGF β must be the basic cause of its effect on wound repair.

Effect of TGF β on cell reorientation

Recent experimental evidence indicates that TGF β controls cell movement in a more complicated manner than

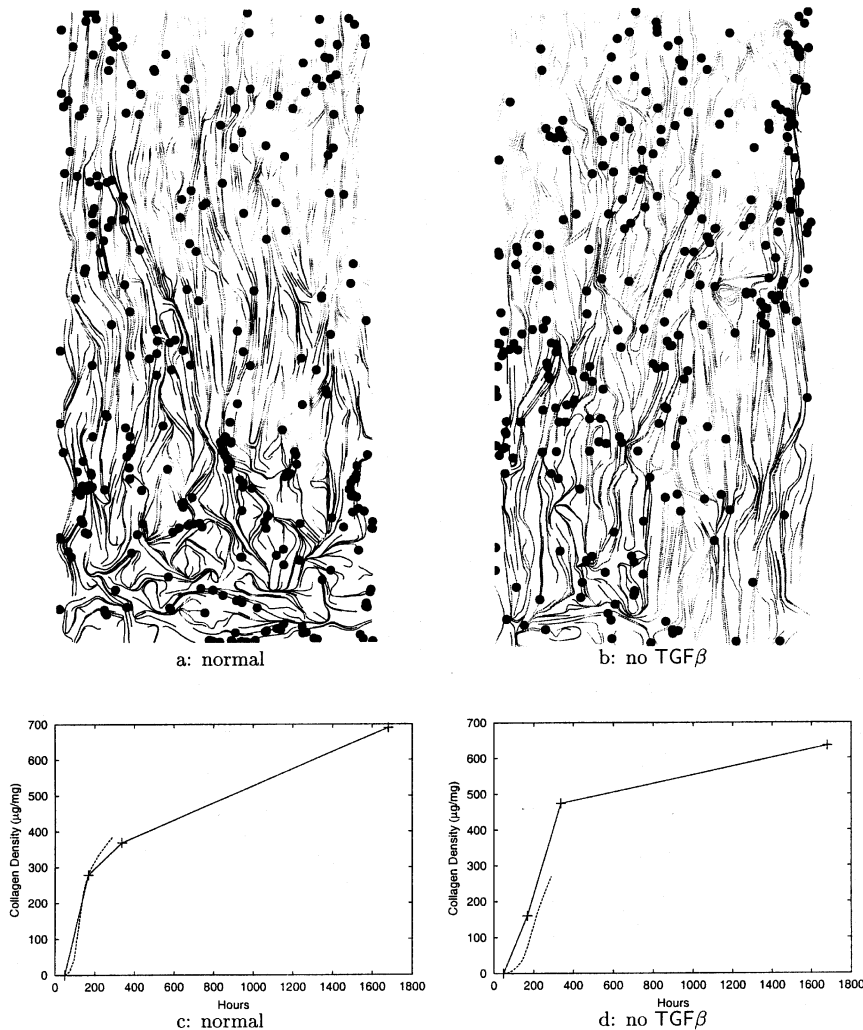


Figure 3. Collagen matrix dynamics for a simulation of normal wound healing with and without TGF β . The collagen matrix for a simulation of normal wound healing with TGF β , resulting in a scar, is shown in (a) with the fibroblast locations marked and graphs of total collagen density for the simulation and the experimental control data taken from Shah et al.¹ are plotted in (c). In (b) we show a simulation of a wound treated with an antibody for TGF β with the corresponding collagen density for the simulation and the experimental data plotted in (d). The anti-TGF β simulation is the same as that shown in Figure 2(a) and removes only the effects of TGF β on cell proliferation, motility and collagen production shown individually in figure Figure 2. The simulation clearly shows that those three effects do not explain the experimental data where anti-TGF β treatment reduces scarring. The collagen matrix is represented after 240 hours of simulation corresponding to about 12 days after wounding (the fibroblasts enter the wound region between 24-48 hours after wounding). Black indicates high density and white indicates no collagen. The line segments are streamlines for the collagen vector field. The location of one fourth of the total fibroblasts are plotted with black circles. The region represents a cross section of a slash wound with the surface of the skin at the top, normal tissue to the right and left and the basement membrane at the bottom. When combining the three effects of TGF β , we assume that the cell proliferation increases at 90 pg/mm³ of TGF β and then decreases for higher concentrations; for the motility we combine the decreased cell speed and the elevated flux at 90 pg/mm³ of TGF β which decreases with increased concentrations of TGF β . The three experimental values in (c) and (d) are denoted by (+) and are averaged values of four observations reported in Shah et al.¹ The simulated collagen density is plotted by the dashed line.

simply controlling the speed of movement. The development of lamellipodia and filopodia by fibroblasts is dependent on TGF β levels, implying control of the frequency with which cells change direction.³¹ There is currently no quantitative experimental data on the extent of this effect, but nevertheless we can study its qualitative implications using the model. Figure 4 shows a simulation where the cells are allowed to reorient three times more frequently than those shown in Figure 3b: approximately every 9 minutes. This significantly alters the resulting alignment, in a manner that agrees with experimentally observed alignment patterns.

Different isoforms of TGF β

Three isoforms of TGF β are found in mammals: TGF β_1 , TGF β_2 and TGF β_3 .²⁹ By neutralizing TGF β_1 and TGF β_2

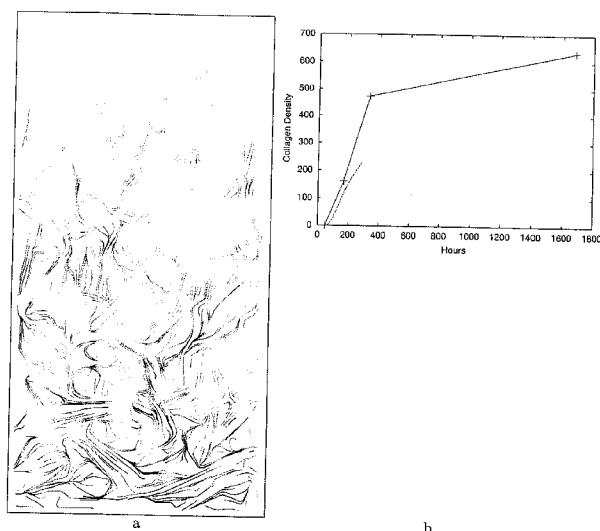


Figure 4. Effect of anti-TGF β_1 treatment on collagen matrix during healing. (a) Represents the collagen matrix for a numerical experiment simulating anti-TGF β_1 treatment, showing a more disordered collagen fiber pattern that is highly reminiscent of experimental data. The more disordered matrix pattern is a key component of reduced scarring. The parameters for this simulation are identical to Figure 3(b) except the cells reorient approximately every 9 minutes instead of every 27 minutes. Graphs of total collagen density for the simulation and from experimental data¹ are plotted in (b). The collagen matrix is represented after 240 hours of simulation corresponding to about 12 days after wounding (the fibroblasts enter the wound region between 24–48 hours after wounding). Black indicates high density and white indicates no collagen. The region represents a cross section of a slash wound with the surface of the skin at the top, normal tissue to the right and left and the basement membrane at the bottom. The line segments are streamlines for the collagen vector field. The three experimental values in (b) are denoted by (+) and are averaged values of four observations reported in Shah et al.¹ The simulated collagen density is plotted by the dashed line.

in a wound or by adding TGF β_3 , scarring is reduced.³ Interestingly, wounds in transgenic mice overexpressing TGF β_1 healed with less scarring;⁴ this finding is consistent with previous results because explicit measurements indicate that in comparison to normal tissue, wound levels are higher for TGF β_3 , and lower for TGF β_1 . Most experimental evidence suggests that the three isoforms affect fibroblasts similarly.^{20,30} There are two notable exceptions: Murata and coworkers³² found that TGF β_3 increases TGF β_1 production and alters collagen production in a manner dependent on TGF β_1 . Incorporating this into our model would not be difficult, but altering the collagen production rate by the magnitude consistent with the experiments would not result in significant differences in alignment. The second exception is recent experimental evidence which suggests that TGF β_3 increases the number of filopodia of mesenchymal cells while TGF β_1 and TGF β_2 promote the formation of lamellipodia.³¹ In this study cells from TGF β_3 knockout mice show a third less filopodia than normal cells from the palatal shelf and when TGF β_3 is added, the knockout cells show 3 to 5 times more filopodia. An increase in the number of filopodia of a cell will cause it to have a better sampling of its environment and will thus tend to increase its probability of moving in other directions. This is because if the filopodia are extended with increased frequency or there are more filopodia for a greater period of time the cell may reorient more frequently. This provides a clear explanation for the anti-scarring effects of TGF β_3 . The experimental data suggests that adding TGF β_3 will tend to cause more frequent cell reorientation, and our mathematical model shows that this will lead to a more disordered network of collagen fibers, which is a hallmark of reduced scarring. Antibodies to TGF β_1 and TGF β_2 , on the other hand, will tend to reduce lamellipodia formation. However, there will also be increased binding of TGF β_3 to the cell surface, since it is likely that to some degree the isoforms bind competitively,^{33,34} and we anticipate that the increase in filopodia formation due to this increased TGF β_3 will be the dominant effect, leading to reduced scarring—again, as observed experimentally.

DISCUSSION

Over the last decade, a number of mathematical models have been developed, to represent different aspects of wound healing. These include models focusing on wound contraction,^{35–37} collagen composition,³⁸ epidermal healing,^{39–41} extracellular matrix alignment,^{42,43} and angiogenesis.^{44,45} Moreover, there is extensive modelling of closely related biological processes, including tissue mechan-

ics,⁴⁶ alignment,^{47,48} and tumor growth and angiogenesis.^{49–51} Although some of this previous work does focus on alignment, the representation of the alignment of dermal tissue in our previous work,¹⁰ on which this model is based, uses novel techniques, in particular the representation of fibroblast cells as discrete objects. In addition, in this paper we model the effects of TGF β on collagen fiber alignment, which to our knowledge has not been done before.

We have presented a mathematical model for scar formation during wound repair that enables a detailed investigation of the effects on collagen fiber orientation of underlying biological processes. By incorporating the role of TGF β into the model, we conclude that its effects on cell proliferation, migration (meaning speed and flux) and extracellular matrix production influence the collagen fiber alignment, which is a key indicator of scar quality,⁹ in only minor ways. Therefore, some other effect is needed to alter alignment if we are to explain its scar reducing properties. We predict that the recently identified regulation by TGF β_3 of filopodia extensions could be the crucial property of the TGF β family, which underlies its ability to regulate scar tissue formation.

Due to the biphasic properties of TGF β , careful time evaluation measurements of this cytokine need to be made and the possibility of significant spatial variation in concentration across the wound must be investigated. For example, we believe that the peak in TGF β concentration five days after wounding is high enough to suppress fibroblast proliferation, while the elevated levels earlier in wounding increase proliferation. This is consistent with experimental evidence,¹⁶ where it was found that cell proliferation decreased dramatically between 5 and 7 days after wounding. The concentrations of TGF β used in most experiments are well below the levels measured in the wound by Yang et al.²²

Figure 4 shows a simulation of the anti-TGF β_1 treatment and one can see that the model predicts much less alignment than for the normal wound healing case, which is shown in Figure 3a. Simulations of adding TGF β_3 are very similar: again there is increased cell reorientation, but the other effects of TGF β (cell proliferation and speed, and collagen production) are retained. The effects of TGF β on cell processes other than reorientation are less significant to alignment, and reorientation is affected differently by the different isoforms, which bind to the cells competitively. Thus our model predicts that it is the ratio of the concentrations of TGF $\beta_{1,2}$ to TGF β_3 that is the key issue in the regulation of fiber alignment within scar tissue. Quantitative data on the dynamics of these isoforms within a healing wound would be key additional information in the further development of the model.

Our model predicts that when the cells reorient every 30 minutes there is pronounced alignment, orientation every 15 minutes gives significant improvement (less alignment) and orientation every 10 minutes gives highly disordered tissue. When more experimental evidence is gathered, quantifying the effects of the TGF β isoforms on orientation, our model could be used to obtain predictions in the form of dose-response curves for the relationship between the concentration of TGF β isoforms and the degree of collagen fiber alignment within scar tissue.

ACKNOWLEDGMENTS

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APPENDIX

In this appendix the model equations used in the simulations are described. For more details the reader is referred to Dallon, Sherratt, Maini.¹⁰ The collagen matrix and fibrin clot (blood clot) are denoted $\mathbf{c}(\mathbf{x})$ and $\mathbf{b}(\mathbf{x})$. These are all vectors in R^2 which depend on time and space. The direction of the vector represents the predominant orientation of the fiber and the length of the vector gives the density of the protein. The fibroblast paths are denoted by \mathbf{f}^i respectively ($i = 1, 2, \dots, M$). The equation determining \mathbf{f}^i is given by

$$\mathbf{f}^i(t)' = s(\|\mathbf{c}(\mathbf{f}^i(t))\|, \|\mathbf{b}(\mathbf{f}^i(t))\|, g) \frac{\mathbf{u}(t)}{\|\mathbf{u}(t)\|} \quad (1)$$

$$\mathbf{u}(t) = (1 - \rho) \frac{\mathbf{v}^i(t)}{\|\mathbf{v}^i(t)\|} + \rho \frac{\mathbf{f}^i(t - \tau)'}{\|\mathbf{f}^i(t - \tau)'\|} \quad (2)$$

$$\mathbf{v}^i(t) = (1 - \alpha)\mathbf{c}(\mathbf{f}^i(t)) + \alpha\mathbf{b}(\mathbf{f}^i(t)). \quad (3)$$

Here prime denotes differentiation with respect to time, g is the concentration of TGF β , s is the speed of the cell and is a function of fiber density and g , τ a time lag and the superscript i identifies the cell. The parameters ρ and α are positive constants. These equations model the contact guidance of the collagen and fibrin matrices as well as the cells directional persistence due to cell polarization. When $\rho = 0$ the cell are not polarized and move only in the direction determined by the extracellular matrix. When $\alpha = 0$ the fibroblast direction is determined entirely by the collagen component of the extracellular matrix. The cell polarization parameter does not dramatically affect the collagen alignment and in all the simulations shown we take $\rho = 0.9$, reflecting the high degree of polarity in fibroblasts on fibrous gels. The value for

α is chosen to be 0.5 assuming that the fibrin clot and the collagen matrix would equally influence the direction of the fibroblasts.

Each fibroblast in the simulation can contribute to the reordering of the collagen. We define a vector \mathbf{f} as

$$\mathbf{f}(\mathbf{x}, t) = \sum_{i=0}^M w(\mathbf{f}^i(t) - \mathbf{x}) \frac{\mathbf{f}^i(t - \tau)'}{\|\mathbf{f}^i(t - \tau)'\|} \quad (4)$$

where τ again represents a time lag. This gives the cumulative affect of all the fibroblasts on the collagen direction with the weight function, w , keeping the influence of the fibroblasts local. The orientation of $\mathbf{c}(\mathbf{x})$ evolves according to

$$\frac{d\theta(t)}{dt} = \kappa \|\mathbf{f}\| \sin(\phi - \theta) \quad (5)$$

where θ is the direction of the collagen matrix at a point in space, κ is a parameter which measures the ability of the fibroblasts to reorder the matrix and ϕ is the direction of the vector \mathbf{f} . This functional form causes the derivative to be small when the difference between the angles is small, maximal when the difference is 90 degrees and it is periodic so when the angles are close to 180 degrees apart the derivative is again small. The orientation of $\mathbf{b}(\mathbf{x})$ is given in the initial conditions. The densities of the proteins are given by the lengths of their representative vectors which are governed by the following ordinary differential equations

$$\frac{d\|\mathbf{c}\|}{dt} = (p_c - d_c \|\mathbf{c}\|) \sum_{i=1}^M w(\mathbf{f}^i - \mathbf{x}) \quad (6)$$

$$\frac{d\|\mathbf{b}\|}{dt} = -d_b \|\mathbf{b}\| \sum_{i=1}^M w(\mathbf{f}^i - \mathbf{x}) \quad (7)$$

where p_c , d_c and d_b are positive functions which depend on the concentration of TGF β . Thus we have assumed the degradation rate for collagen and fibrin which is proportional to the corresponding protein density.

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