A mathematical model for collagen fibre formation during foetal and adult dermal wound healing

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SUMMARY

Adult dermal wounds, in contrast to foetal wounds, heal with the formation of scar tissue. A crucial factor in determining the nature of the healed tissue is the ratio of collagen 1 to collagen 3, which regulates the diameter of collagen fibres. We develop a mathematical model which focuses on the stimulus for collagen synthesis due to the secretion of the different isoforms of the regulatory chemical transforming growth factor β. Numerical simulations of the model lead to a value of this ratio consistent with that of healthy tissue for the foetus but corresponding to scarring in adult wound healing. We investigate the effect of topical application of TGFβ isoforms during healing and determine the key parameters which control the difference between adult and foetal repair.

1. INTRODUCTION

The process of dermal wound healing involves the complex interaction of many cell types and occurs as a sequential cascade of overlapping processes. In adult wound repair, in contrast to foetal healing, the end result is scar formation. This initially reddish, slightly elevated scar gradually turns pale and becomes slightly recessed. It is less functional than the surrounding uninjured tissue because of the lack of a number of key components (Rudolph *et al.* 1992).

In response to injury, fibroblasts migrate into the wound domain, from the surrounding unwounded dermal tissue and from the underlying subcutaneous tissue, the exact source of fibroblasts being an area of much biological controversy. The fibroblasts synthesize chains of amino acids called procollagens (McDonald 1988), a process which is activated by growth factors, including in particular type β transforming growth factor (TGFβ) (Appling *et al.* 1989). Biologically inactive (latent forms of TGFβ isoforms are secreted by many cells (Martin *et al.* 1992; Streuli *et al.* 1993), are autoinductive and have a considerably longer half-life than the active forms (Roberts & Sporn 1990). The wound site contains enzymes which activate latent growth factors and also initiate the stabilization of collagen precursors (Miller & Gay 1992). In human skin, collagenase is synthesized and secreted by fibroblasts as a 'zymogen' (Stricklin *et al.* 1978), but collagen degradation cannot occur until the zymogen is activated.

Dermal tissue contains two main types of collagen, types I and III. Type III collagen decorates the surface of the type I collagen fibril so that a higher ratio of type III to type I results in thinner fibres (Whitby & Ferguson 1991). Another key difference between scar and normal tissue is collagen fibre orientation. In normal skin, the collagen fibres in the dermis exhibit a basketweave-like arrangement. In scar tissue, the fibres are longer and thinner than in normal tissue, because of higher levels of type III collagen (Mast *et al.* 1992), and the fibres are orientated

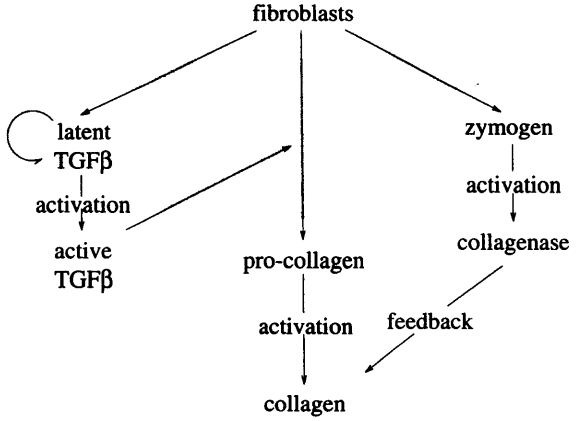

Figure 1. Schematic representation of the interactions between growth factors, proteins, fibroblasts and enzymes during the wound healing process. The fibroblast cells secrete precursors to both collagen and collagenase, so that there is a feedback control loop. They also secrete growth factors which regulate collagen production, again in a precursor (latent) form. The various precursor forms are made active via enzymes which are released as part of the response to wounding, and are absent in unwounded skin. It is this feature of the system that results in a continuous range of possible healed states. Our full model equations contain variables reflecting the presence of two isoforms of TGFβ, two types of collagen and correspondingly two types of zymogen.

in the direction of the tension (Peacock 1984). There is some recent evidence that other collagen types may be expressed during wound healing and influence scar quality (Hopkinson *et al.* 1995); however we will neglect these, and focus entirely on the dominant collagen types, I and III.

Experimental understanding of foetal wounds has increased dramatically over the past 15 years and this has led to the possibility of improved adult healing. Foetal and postnatal mammalian wounds have been shown to heal differently, the main difference being that foetal wounds heal without scar formation; during the late foetal stages and early childhood, there is a gradual transition to the adult response to injury, namely repair by scar tissue (Adzick & Longaker 1992). This absence of scarring is reflected in a number of molecular differences between healed adult and foetal wounds, notably the diameter of collagen fibres and their orientation. In this paper, we focus on the first of these, and develop a mathematical model which addresses the biochemical mechanisms responsible for the difference in fibre thickness, and its implications for chemical reduction of adult scarring.

2. MATHEMATICAL MODELLING

In this section, we derive a detailed mathematical model for the formation of collagen fibres during foetal dermal wound healing. We neglect the important issue of orientation, to focus on the ratio of type I to type III collagen, which controls fibre thickness. Collagen is secreted during wound healing by fibroblast cells. There is a current controversy concerning whether fibroblasts migrate into the wound from the underlying subcutaneous tissue or from the neighbouring dermis. In the former case, spatial variations within the wound will be quite small and thus we can reasonably consider a purely temporal model. We will adopt such a spatially homogeneous framework, which reflects the synthesis by fibroblasts of collagen, TGFβ, and the collagen degrading collagenases, and we introduce both latent and active forms of the model variables, with generic enzymes for activation.

Fibroblasts, $f(t)$, are the main cell type in the dermis. In the absence of TGFβ, the cell population increases exponentially at low densities but saturates for high cell densities and we thus model fibroblast proliferation by a chemically enhanced logistic growth term. We assume that normal dermal fibroblasts die at a constant rate, A_4. Hence the equation for fibroblast density is

$$\frac{\mathrm{d}f}{\mathrm{d}t} = \overbrace{(A_1 + A_2\beta_1 + A_3\beta_3)f\left(1 - \frac{f}{k_1}\right)}^{\text{mitotic generation}} - \overbrace{A_4 f}^{\text{natural loss}}.$$

In our model, we represent active TGFβ via the variables $\beta_1(t)$ (representing isoforms 1 and 2) and $\beta_3(t)$ (representing isoform 3). The fibroblasts are stimulated via autocrine regulation (Roberts & Sporn 1990) to secrete the corresponding latent TGFβs, $l_1(t)$ and $l_3(t)$; the production rate saturates at high growth factor concentrations (Wakefield *et al.* 1988). Latent TGFβ also undergoes an autocrine mechanism, whereby TGFβ induces self-secretion. Latent TGFβ has a short half-life and we model natural decay as a first order process (Wakefield 1990). The concentration of latent growth factor is also decreased because of activation by specific enzymes. These various effects lead to the equations

$$\frac{\mathrm{d}l_1}{\mathrm{d}t} = \overbrace{A_5 f l_1/(1 + A_6 l_3 + A_7 l_1)}^{\text{production by fibroblasts}} - \overbrace{A_8 l_1}^{\text{natural decay}} - \overbrace{A_{16} e_1 l_1}^{\text{activation}}$$

$$\frac{\mathrm{d}l_3}{\mathrm{d}t} = A_9 f l_3/(1 + A_{10} l_3) - A_{11} l_3 - A_{17} e_1 l_3.$$

Fibroblast proliferation and collagen synthesis are upregulated by TGFβs, but by active rather than latent forms (Krummel *et al.* 1988). The latent forms of TGFβ are activated by specific enzymes, and experiments have shown that active TGFβs, $\beta_1(t)$ and $\beta_3(t)$, undergo rapid decay, which we model as a first order process for both isoforms (Roberts & Sporn 1990).

$$\frac{\mathrm{d}\beta_1}{\mathrm{d}t} = \overbrace{A_{12} e_1 l_1}^{\text{activation}} - \overbrace{A_{13}\beta_1}^{\text{natural decay}}$$

$$\frac{\mathrm{d}\beta_3}{\mathrm{d}t} = A_{14} e_1 l_3 - A_{15}\beta_3.$$

The enzyme $e_1(t)$ is one of three generic enzymes in our model formulation. During the inflammation stage, white blood cells release a range of enzymes which activate growth factors, procollagens and zymogens (Sinclair & Ryan 1994). These pools of enzyme are rapidly degraded during the healing process. Because detailed descriptions of the various enzymes are not currently available, we represent their effects by generic enzymes $e_1(t)$, $e_2(t)$, $e_3(t)$, and use the law of mass action to model the activation of latent TGFβ1 and 3 and type I and type III collagen and collagenases:

$$\frac{\mathrm{d}e_1}{\mathrm{d}t} = \overbrace{-e_1(A_{16} l_1 + A_{17} l_3)}^{\text{activation of latent forms}},$$

$$\frac{\mathrm{d}e_2}{\mathrm{d}t} = -e_2(A_{18} p_1 + A_{19} p_3),$$

$$\frac{\mathrm{d}e_3}{\mathrm{d}t} = -e_3(A_{40} z_1 + A_{41} z_3).$$

Here $p_1(t)$ and $p_3(t)$ denote the concentrations of procollagens I and III, and $z_1(t)$ and $z_3(t)$ are the corresponding zymogens. Procollagen is synthesized by fibroblasts, in response to injury (McDonald 1988) and we focus on two types of procollagens, types I and III. In the absence of chemicals, we assume a constant secretion rate, but experiments show upregulation of procollagen synthesis by active TGFβ (Appling *et al.* 1989), hence the inclusion of a linear function of the active chemical concentrations. We assume procollagen fibres have a constant life-span and model natural decay as a first order process:

$$\frac{dp_1}{dt} = \overbrace{(A_{20}+A_{21}\beta_1+A_{22}\beta_3)f}^{\text{secretion by fibroblasts}} - \overbrace{A_{23}p_1}^{\text{natural decay}} - \overbrace{A_{18}e_2p_1}^{\text{conversion by enzyme}}$$

$$\frac{dp_3}{dt} = (A_{24}+A_{25}\beta_1+A_{26}\beta_3)f - A_{27}p_3 - A_{19}e_2p_3.$$

Collagen I and III are triple helical structural proteins, formed by the activation of procollagens I and III respectively. We use the law of mass action to model the activation of procollagen fibres to collagen fibrils, $c_1(t)$ and $c_3(t)$. Collagenases I and III gradually degrade collagen I and III, respectively. There is little experimental evidence for the processes involved in the degradation, and thus for simplicity we assume a linear form:

$$\frac{dc_1}{dt} = \overbrace{A_{28}p_1e_2}^{\text{activation}} - \overbrace{A_{29}s_1c_1}^{\text{degradation}}$$

$$\frac{dc_3}{dt} = A_{30}p_3e_2 - A_{31}s_3c_3$$

The zymogens are the inactive forms of collagenase, secreted by fibroblasts, but the secretion is inhibited by the presence of active TGFβ (Jeffrey 1992). The natural decay of zymogens is taken to be first order.

3. SOLUTIONS OF THE MODEL

The model equations have two steady states; the unstable wounded state, with everything zero, and the stable healed steady state, with all the variables at their normal dermal level (i.e. $f=f^0$, $l_1=l_1^0$, $l_3=l_3^0$, $e_1=e_2=e_3=0$, $s_1=s_3=0$) except for collagen I and III, which can take any value ($c_1=c_1^*$, $c_3=c_3^*$). This is a crucial property of the model, because experimental results suggest that the final healed levels of collagen I and III are altered by transient exogenous applications of TGFβs.

To investigate the temporal changes in the wound milieu, we solve the tenth order system of ordinary differential equations numerically using a Runge–Kutta–Merson method. We require a small number of fibroblasts and a low concentration of growth factors to initiate the healing process and we thus impose the

$$\frac{dz_1}{dt} = \overbrace{\frac{A_{32}}{1+A_{33}\beta_1+A_{34}\beta_3}fc_1}^{\text{secretion by fibroblasts}} - \overbrace{A_{35}z_1}^{\text{natural decay}} - \overbrace{A_{40}e_3z_1}^{\text{activation}}$$

$$\frac{dz_3}{dt} = \frac{A_{36}}{1+A_{37}\beta_1+A_{38}\beta_3}fc_3 - A_{39}z_3 - A_{41}e_3z_3.$$

Collagenases I and III, $s_1(t)$ and $s_3(t)$, are the active form of zymogens, which bind avidly to collagen I and III, breaking down the fibres. We hypothesize that the collagenases arise via enzymatic activation, independent of TGFβ. Therefore, we have

$$\frac{ds_1}{dt} = \overbrace{A_{42}z_1e_3}^{\text{activation of zymogen}} - \overbrace{A_{43}s_1}^{\text{natural degradation}}$$

$$\frac{ds_3}{dt} = A_{44}z_3e_3 - A_{45}s_3.$$

Experimental data suggest that the degradation of active TGFβ occurs on a much faster timescale than that of latent TGFβ (Robert & Sporn 1990), and thus we assume that the concentrations of active TGFβ1 and 3 are always in equilibrium. Using a similar argument, we assume that the procollagens and zymogens are both in equilibrium and hence the number of equations is reduced from 16 to ten.

The estimation of dimensional parameter values is essential for biologically realistic model predictions. The governing equations contain a large number of parameters whereas there is a limited source of experimental data. However, we know the timescale of the healing process and can hence obtain order of magnitude estimates for some of the remaining parameters. The values we use, with brief explanations, are given in the legend to figure 2, with further details in the remainder of the text.

initial conditions shown in figure 2. The initial conditions correspond to a few fibroblasts and growth factors scattered uniformly throughout the wound, and a pool of generic enzymes released by the initial inflammatory response to wounding. Extensive numerical solutions suggests robustness for a large range of initial amounts of fibroblasts and growth factors.

(*a*) *Normal foetal healing*

Figure 2 shows the time evolution of the model variables during a simulation of foetal healing. There is a rapid initial increase of fibroblasts and TGFβ, before they settle down to the normal dermal levels, in agreement with experimental observations (Ferguson & Howarth 1992). Enzyme 1 gradually decreases with time whereas enzymes 2 and 3 undergo a much more rapid decay. We use these solutions to fix the degradation parameters A_{18}, A_{19}, A_{40} and A_{41} and use experimental observations to choose A_{21}, A_{22}, A_{25} and A_{26}. The parameters A_{28} and A_{30} are fixed by comparing model solutions with experimental results, to obtain the required collagen ratio. The new levels of collagen I and III are quickly attained and maintained throughout the healing process in a ratio of about 3:1. This is not a prediction of the model: rather, we have used this experimental result in determining model parameter values. We have then investigated how this

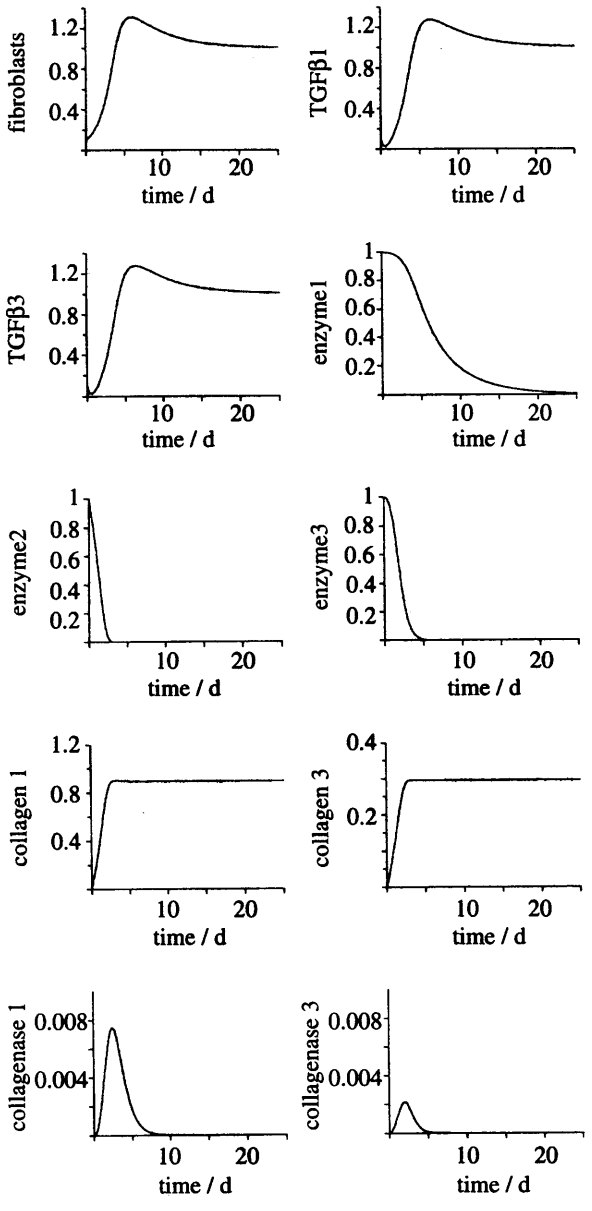

Figure 2. Numerical solution of the model equations for the 20 days post-wounding, for parameter values corresponding to foetal skin. We impose the initial conditions corresponding to small levels of fibroblasts and latent growth factors, and high initial concentrations of our three generic enzymes, which are produced as a response to wounding. We assume that the collagens and collagenases are initially absent in the wound. The fibroblasts and chemical regulators rapidly attain their steady state values and the collagen I and III densities evolve so that their final healed ratio is approximately 3:1, as in normal foetal skin. Specifically, we take as initial conditions $f(0) = l_1(0) = l_3(0) = 0.1 \quad e_i(0) = 1$, $i = 1, 2, 3, c_1(0) = c_3(0) = s_1(0) = s_3(0) = 0$. Here and in the figure, we express f, l_1, l_3 as a proportion of the corresponding unwounded levels, which we denote by f^0, l_1^0 and l_3^0; we estimate these parameters as $f^0 = 10^5$ ml^{-1} (Morgan & Pledger 1992), and $l_1^0 = l_3^0 = 2$ ng ml^{-1} (Streuli *et al.* 1993). The collagen densities c_1 and c_3 are expressed as a proportion of the unwounded level of collagen I in normal foetal dermis, which is approximately $c_1^0 = 12.5$ μg mg^{-1} dry weight (Merkel *et al.* 1988). The enzyme concentrations e_i are expressed as a proportion of the initial levels, e_i^0 say, and collagenase levels are expressed as a proportion of the maximum levels observed on wounding, denoted s_1^0. The following dimensionless parameter ratios were used in the numerical solution illustrated, with the time scale T taken as 1 d; we give references in cases in which we have been able to base our parameter estimates on firm experimental data: $A_1/T = 1.4$, $A_2 A_{12} e_1^0 l_1^0/(A_{13} T) = 0.5$ (Roberts & Sporn 1990), $A_3 A_{14} e_1^0 l_3^0/(A_{15} T) = 0.5$ (Roberts & Sporn 1990), $A_4/T = 0.7$ (Ferguson & Howarth 1992), $A_5 f^0/T = 100$, $A_6 l_3^0 = 5$, $A_7 l_1^0 = 4$, $A_8/T = 10$ (Wakefield 1990), $A_9 f^0/T = 100$, $A_{10} l_3^0 = 9$, $A_{11}/T = 10$ (Wakefield 1990), $A_{16} l_1^0/T = 0.1$, $A_{17} l_3^0/T = 0.1$, $A_{18} A_{20} f^0/T^2 = 3$, $A_{19} A_{24} f^0/T^2 = 3$, $A_{18} A_{21} A_{12} f^0 e_1^0 l_1^0/(A_{13} T^2) = 1.5$, $A_{18} A_{22} A_{14} f^0 e_1^0 l_3^0/(A_{15} T^2) = 3.5$, $A_{23}/T = 1$, $A_{25} A_{19} A_{12} f^0 e_1^0 l_1^0/(A_{13} T^2) = 1.5$, $A_{26} A_{19} A_{14} f^0 e_1^0 l_3^0/(A_{15} T^2) = 2$, $A_{27}/T = 1$, $A_{28} A_{20} f^0 e_2^0/(c_1^0 T^2) = 5.4$, $A_{29} s_1^0/T = 1$, $A_{30} A_{24} f^0 e_2^0/(c_1^0 T^2) = 1.8$, $A_{31} s_1^0/T = 1$, $A_{32} A_{42} f^0 c_1^0 e_3^0/(T^2 s_1^0) = 1.5$, $A_{33} A_{12} e_1^0 l_1^0/A_{13} = 1$, $A_{34} A_{14} e_1^0 l_3^0/A_{15} = 3$, $A_{35}/T = 10$, $A_{36} A_{44} f^0 c_1^0 e_3^0/(T^2 s_1^0) = 4.5$, $A_{37} A_{12} e_1^0 l_1^0/A_{13} = 3$, $A_{38} A_{14} e_1^0 l_3^0/A_{15} = 1$, $A_{39}/T = 10$, $A_{40} A_{32} f^0 c_1^0/T^2 = 50$, $A_{41} A_{36} f^0 c_1^0/T^2 = 50$, $A_{43}/T = 1$, $A_{45}/T = 1$, $k_1/T = 2$ (Morgan & Pledger 1992).

predicted ratio changes when parameters are altered to reflect healing in adult rather than foetal skin.

(*b*) *Adult healing*

We adapt our model for foetal healing by changing some of the parameters based on experimental evidence. The main differences are that the cell cycle time for adult fibroblasts is much slower than in the foetal case and the unwounded concentrations of TGFβ1 and TGFβ3 in adult dermal tissue are 80 and 40 ng ml^{-1} respectively (Roberts & Sporn 1990), a 20-fold increase on the corresponding foetal levels. The total collagen content in normal adult dermal tissue is also about twenty-fold higher, at 331 μg per mg dry weight (Merkel *et al.* 1988), 15% of which is type III. We thus estimate new parameter values, as given in the legend to figure 3.

We solve the system of ordinary differential equations with these amended parameter values, and with the same initial conditions of a few fibroblasts and growth factors uniformly scattered throughout the wound. The level of fibroblasts, TGFβ1 and TGFβ3 increase initially but settle down, after the realistic repair time of about 60 d, to their normal dermal levels. The collagenases are gradually degraded to zero while the collagens rapidly attain their healed levels (figure 3*a*), in which the ratio of collagen I:III is about 3.6:1, in fair agreement with the experimentally determined ratio of 4:1.

(*c*) *Addition of TGFβ*

Much experimental work has been down on the effects of the addition of various growth factors on wounds, mainly adult wounds. Dermal adult rat wounds injected at the margin with neutralizing antibody (NA) to TGFβ1 have been shown to heal with reduced or no scarring, and with identical tensile strength and more normal dermal architecture than untreated wounds. The reduced scarring in the NA treated wounds was not accompanied by a delay in wound healing or a reduction in wound strength (Shah

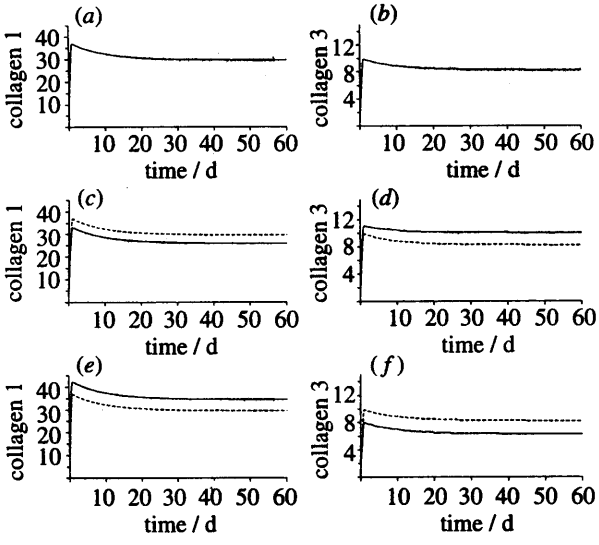

Figure 3. Numerical solution of the model equations for the 60 days post-wounding, for parameter values corresponding to adult skin. As in figure 2, we impose the initial conditions corresponding to small levels of fibroblasts and latent growth factors, and high initial concentrations of our three generic enzymes, which are produced as a response to wounding, and we assume that the collagens and collagenases are initially absent in the wound. The fibroblasts and chemical regulators rapidly attain their steady state values (not shown). We plot the solutions only for collagen type I and III densities, which are expressed as a proportion of c_1^0, the density of collagen I in unwounded foetal dermis. The parameters are as in figure 2, except for the following which we have altered to reflect the differences between adult and foetal skin, as discussed in the text: $A_1/T = 0.332$, $A_4/T = 0.166$ (Morgan & Pledger 1992), $A_6 l_3^0 = 0.255$, $A_7 l_1^0 = 0.1$, $A_{10} l_3^0 = 0.45$ (Roberts & Sporn 1990), $A_{28} A_{20} f^0 e_2^0/(c_1^0 T^2) = 202.5$, $A_{30} A_{24} f^0 e_2^0/(c_1^0 T^2) = 67.5$ (Merkel *et al.* 1988). In (a, b) no TGFβ is added exogenously and the ratio of collagen types I and III is 3.6:1, implying thinner, more densely packed fibres than in normal skin (ratio about 5.7:1), as is observed experimentally in scar tissue. Figures (c, d) and (e, f) correspond to addition of TGFβ1 and 3, respectively, which are reflected in the model by a change in the respective TGFβ initial condition. Otherwise the initial conditions in (a, b), (c, d) and (e, f) are as in figure 2. The final healed ratios of collagen I:III are 2.57:1 (thinner collagen fibres) and 5.67:1 (thicker collagen fibres), respectively. These changes are consistent with experimental data. The dashed lines in (c, d) and (e, f) are a reproduction of the solution from (a, b), to aid comparison.

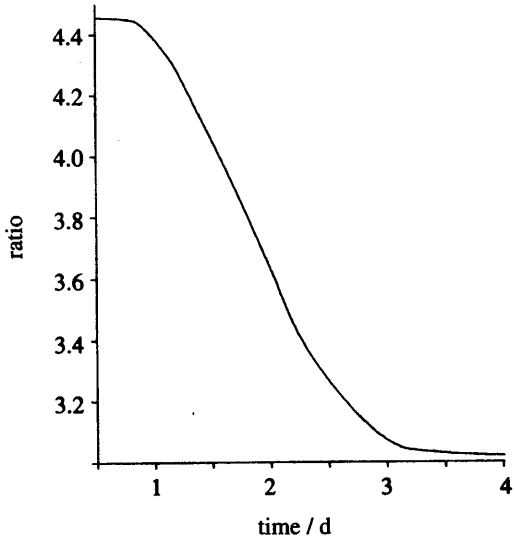

Figure 4. The change in the ratio of collagen I and III with the time at which a fixed concentration of TGFβ1 is added to a foetal wound during healing. The parameters and initial conditions are as in figure 2. The addition of growth factor is modelled by interrupting the numerical solution at one point during healing, and continuing with new initial conditions for l_1 or l_3. There is a sharp decrease in the final healed ratio (corresponding to thinner fibres and poorer healing) as the time of application increases, so that by about day 2 post-wounding, addition of growth factor has little effect on the final ratio.

et al. 1992). Research has also shown that when either TGFβ1 or TGFβ3 is added exogenously to foetal wounds in rabbits (Krummel *et al.* 1988), the final ratio of the collagens changes. In particular, it has been shown that topical applications of TGFβ1 increases the amount of collagen III relative to collagen I.

To model the addition of TGFβ in our system, we simply alter the initial conditions to give high initial levels of l_1 or l_3. By varying these initial concentrations, numerical simulations indicate that the ratio of the collagen I to III is changed in both adult and foetal healing.

For the foetal case, our model predicts a saturating concentration of 0.4 μg ml^{-1}, beyond which addition of more chemical has a negligible effect on the ratio of collagens. The concentration is in agreement with the order of magnitude of the concentrations of chemical applied topically in the experiments (Krummel *et al.* 1988). Numerical solutions with the addition of TGFβ1 indicate new steady state levels of collagen I and III. There is an enhanced ratio of 3:2 and a reduction in the total amount of collagen, implying that the collagen fibrils in the healed wound are much thinner in diameter than in normal foetal wounds, which is a characteristic of scarring. Similarly, by changing the initial conditions to model the addition of TGFβ, we find the ratio of collagen I to III increases, corresponding to thicker fibres. This may correspond to either high quality healing or possibly abnormalities such as hypertrophic scarring (Skalli & Gabbiani 1988; Olsen *et al.* 1996).

We have also investigated the importance of the time at which TGFβ is added to wounds, by simulating the addition of 0.4 μg ml^{-1} of TGFβ3 at a variety of points during the healing process. Our results indicate that early addition is essential. In fact, addition of growth factor after day 2 of healing has little effect on either the density of collagen or the ratio of types I to III (figure 4).

For the adult case, addition of TGFβ1 results in final levels of collagen I and III in a ratio of about 2.6:1, implying much thinner fibres (figure 3*b*). Shah *et al.* (1992) showed that when TGFβ is applied topically to an adult wound immediately after wounding, there is a transient increase in the total collagen level but that after 14 d it is of a similar magnitude to the healed level. The phenomenon of an initial increase in collagen is observed in our numerical solutions and the final

collagen levels correspond to fibre diameters that agree well with experimental results. In figure 3*c* we have simulated the addition of TGFβ3 and observe collagen levels in the new ratio of 5.6:1 corresponding to 15% type III collagen. Again it should be noted that early addition of TGFβ is essential, with little effect for application after day 15. We thus predict that addition of TGFβ3 reduces scarring, with the healed tissue having a similar percentage of type III collagen as normal dermal tissue.

(*d*) *The difference between TGFβ1 and TGFβ3*

An important biological feature that is included in our model is the modulation of latent TGFβ1 by latent TGFβ3, a phenomenon which recent experiments suggest is not mimicked in the corresponding TGFβ3 equation. This is represented by the inclusion of the term $A_6 l_3$ in the latent growth factor equations. We investigate the importance of this term by numerically solving the system of ordinary differential equations with $A_6 = 0$, corresponding to no modulation. In the foetal case, neither the final levels of collagen I and III nor the ratio are significantly altered by the removal of this term, indicating that the modulation of TGFβ1 is not crucial for foetal dermal wound healing. However, numerical solutions for adult wound healing indicate that the final ratio of collagen I to III is decreased by about 30% following the removal of this term. This is as expected intuitively because the steady state level of TGFβ1 is increased and this has already been shown to produce thinner fibres. Further simulations reveal that for addition of TGFβ3, the saturating levels for collagen I and III and the final ratio are also decreased. Hence, we conjecture that the modulation of TGFβ1 by TGFβ3 is not significant in foetal repair, but plays a key role in adult wound healing, because the intrinsic growth factor levels are higher. Alteration of this modulatory effect may be a viable alternative to the addition of growth factors as a method for reducing scar tissue formation in adult dermal wounds.

(*e*) *Key parameters*

Numerical solutions of the model suggest healed levels of collagen which are in agreement with experimental results. However, a large number of the model parameters are either unknown or fixed by comparing numerical solutions with experimental data. We thus consider the robustness of the model to changes in parameter space. This enables us to determine the key parameters which have the greatest influence on the final levels of collagen, so that we can hypothesize as to which parameters account for the differences between adult and foetal wound healing.

Our method of parameter sensitivity analysis is to alter a parameter by a given percentage, and to compare the relative change in the Euclidean norm of the solution with that in the parameter. We make this comparison at a number of time points, and we alter each parameter in turn, except where two parameters must be changed in parallel to preserve the steady state structure of equations. (For example, if the fibroblast proliferation rate A_1 is altered, then a corresponding change to the loss rate A_4 is required so that the unwounded steady state does not change.) We have considered parameter changes of up to ±50%, and our results indicate that the model is in fact very robust to changes in almost all parameter values. The greatest sensitivity is to parameters A_8 and A_{11}, the natural decay rates of latent TGFβ1 and 3 respectively, and A_{28} and A_{30}, the rates of activation of procollagen I and III to produce collagen I and III. Varying the parameters A_8 and A_{11} causes a change of less than 2% in final collagen levels, with the ratio being maintained at 3:1, but the healing time is altered significantly: for example, a parameter decrease/increase of 50% causes a 20% decrease/increase in healing time. Moreover, changes in A_{28} and A_{30} result in new healed levels for types I and III collagen, and a new ratio. For example, halving the value of A_{28}, with all the other parameters unchanged, results in a 20% decrease in the collagen I steady state level and thus a change in ratio. We can thus conclude from numerical solutions that it is these two parameters that crucially affect the quality of healing. It should be noted that there is little experimental data for these parameter values, and their determination is thus an important experimental goal. Furthermore, parameter sensitivity analysis for the adult model indicates that A_6, the parameter for the modulation of TGFβ1 by TGFβ3, is also crucial, as discussed above.

4. DISCUSSION

Scar tissue formation following trauma or surgery is a significant clinical problem, often resulting in defective growth or functional impairment. Adult wound healing entails a complex series of events, involving many cell types and chemicals, ultimately ending in scar formation. In contrast, foetal wounds heal more rapidly, with complete regeneration of the tissue and no scar. The ultimate aim of foetal wound healing studies is thus to be able to manipulate the adult wounds so that they heal in a scarless, foetal-like, fashion. Scar tissue is defined in terms of the density, diameter and orientation of the collagen fibres within the wound domain. In adult wounds, thinner, more densely packed collagen fibres are observed, with the fibres being oriented along the lines of tension, in contrast to the basket-weave orientation of normal tissue. The diameter of the collagen fibrils is related to the ratio of types I to III, with higher levels of type III collagen resulting in thinner fibres. Here, we have presented a model which aims to explain one of the key differences between adult and foetal repair, namely the final levels and the ratio of collagens I and III.

In this paper, we have concentrated on TGFβ1 and 3 as the important regulators of collagen fibril levels. We now briefly discuss other factors which play some role in the healing process. Platelet derived growth factor (PDGF) is a glucoprotein which is released by platelets in abundance at the time of wounding. However, research has shown that the effect of PDGF

on collagen deposition is small compared to the effect of TGFβ (Pierce *et al.* 1991) and we thus choose to neglect it in our model. Fibroblast growth factor (FGF) can act as an angiogenic factor *in vivo* and *in vitro*, and the basic form triggers normal granulation tissue formation, with the secretion of fibronectin and collagen. However, experiments indicate that FGF most significantly enhances neovascularization and re-epithelialization (reviewed by Martin *et al.* 1992) and again we can legitimately neglect its effect on collagen in comparison to TGFβ. Fibronectins are complex glycoprotein matrix molecules, which act as a 'scaffold' for collagen deposition (Whitby & Ferguson 1992); granulation tissue fibroblasts are coated with a layer of fibronectin matrix and myofibroblasts are covered with fibronectin which attaches to stress fibres in the wound (McDonald 1988; Clark 1989). Inclusion of fibronectin is one possible extension to the model, but as it primarily effects the arrangements of fibres and not the density or diameter, we do not anticipate that this would significantly alter our results. Finally we mention that our model excludes many other features such as mechanical loading, which may, in certain cases, have significant effects on wound quality.

In summary, our model for foetal wound healing predicts levels of collagen I and III which compare favourably with experimental data, both in normal healing and when TGFβ1 is applied topically to the wound. The model also predicts that early addition of TGFβ3 results in thicker collagen fibres, a result which could be tested experimentally. In the adult case, numerical solutions suggest healed levels of collagen I and III which correspond to scarring in normal healing, but an early addition of TGFβ3 reduces the ratio of collagen I to III, thus improving wound quality.

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