Modeling the effects of treating diabetic wounds with engineered skin substitutes

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ABSTRACT

In this paper, a novel mathematical model of wound healing in both normal and diabetic cases is presented, focusing upon the effects of adding two currently available commercial engineered skin substitute therapies to the wound (Apligraf™ and Dermagraft™). Our work extends a previously developed model, which considers inflammatory and repair macrophage dynamics in normal and diabetic wound healing. Here, we extend the model to include equations for platelet-derived growth factor concentration, fibroblast density, collagen density, and hyaluronan concentration. This enables us to examine the variation of these components in both normal and diabetic wound healing cases, and to model the treatment protocols of these therapies. Within the context of our model, we find that the key component to successful healing in diabetic wounds is hyaluronan and that the therapies work by increasing the amount of hyaluronan available in the wound environment. The time-to-healing results correlate with those observed in clinical trials and the model goes some way to establishing an understanding of why diabetic wounds do not heal, and how these treatments affect the diabetic wound environment to promote wound closure.

The wound healing process in acute wounds is well understood and documented, but in chronic wounds, such as diabetic ulcers, the process of healing is disrupted and the exact nature of the healing failure is not known. Recent advances in wound healing therapies have resulted in the development of treatments used to successfully close diabetic wounds, even those that have not responded to conventional wound management strategies.[1–4] The question that therefore arises is this—given that the exact mechanism by which diabetic wounds fail to heal is not known, how do these advanced therapies work? The aim of this study is to suggest answers to this question using theoretical modeling, and to predict ways in which the therapies could be optimized.

One feature of diabetic wounds is that they often appear to have a persistent and excessive number of macrophages,[5] and moreover the balance between inflammatory and repair macrophages is disrupted.[6] In order to address the question of how these therapies work, we first need to understand why diabetic wounds do not heal. This article investigates further the hypothesis that diabetic wounds do not heal because they do not progress past the inflammatory stage of wound healing.

The model simulates the wound healing process, starting with the inflammatory and repair macrophages. These cells produce growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), which then attract other cells such as fibroblasts and keratinocytes to the wound site. Fibroblasts in turn produce extracellular matrix (ECM) components such as collagen and hyaluronan to form granulation tissue and so encourage wound repair.[7] Through the use of this model, which concentrates on the interactions between the growth factors, cell populations, and ECM components, we can manipulate this wound healing system in a way that is not possible in a laboratory or clinical setting, and hence gain a more comprehensive understanding of chronic wound healing dynamics. Within the context of our model, we can investigate the effects of adding single- and multicomponent treatments to diabetic wounds.

MATERIALS AND METHODS

The model equations used are ordinary differential equations and were derived specifically for this model using a conservation approach. The equations for the inflammatory macrophage, repair macrophage, and fibroblast populations were determined using

$$\text{Rate of change of cell population} = \text{cell migration} + \text{cell mitosis} - \text{cell death}.$$

Similarly, the equations for TGF-β, PDGF, collagen, and hyaluronan were determined using

$$\text{Rate of change of chemical} = \text{chemical production} - \text{chemical decay}.$$

These broad conservation equations were then used to derive specific equations for each of the model variables. The actual equations used, together with the parameter values for each variable and forms of migration functions, are presented in more detail in Appendix A. The equations were solved using the ordinary differential equation numerical solution subroutine ODE15s of the MATLAB computing package over a time period of 200 days. This time period was chosen as this time period showed that all variables had reached equilibrium—shorter time periods showed that the

system was still moving toward equilibrium conditions. The initial conditions used are the same for both normal and diabetic wound repair, $\Phi_I(0)=\varphi_R(0)=200\,\text{cells/mm}^3$, $T(0)=6\,\text{pg/mm}^3$, $P(0)=2\,\text{pg/mm}^3$, $F(0)=50\,\text{cells/mm}^3$, $C(0)=10\,\mu\text{g/mm}^3$, and $H(0)=0.01\,\mu\text{g/mm}^3$. These initial conditions were estimated from studies reporting on wound healing.[5,7–12] The differences between the normal and diabetic cases are captured in the parameter values for the fibroblast production rate of collagen, k_{11}, the fibroblast production rate of hyaluronan, k_{12}, and the fibroblast death rate, d_4. All other parameter values (as given in Table A1) remain the same for both cases. The basis for these differences in parameter values are indicated in the literature.[13–17]

When modeling the treatment protocols for two currently available commercial engineered skin substitutes, Apligraf™ and Dermagraft™, it is assumed that each treatment application is modeled as being applied at a particular time point and at that time point all the components of the treatment are applied to the wound.

The model used is an extension of one previously presented,[12] which investigated the behavior of three components: inflammatory macrophages, repair macrophages, and TGF-β. The initial model showed that diabetic wounds have a higher TGF-β level than normal wounds, and that macrophages persist longer in diabetic wounds than in normal wounds. In this extended model, we add equations for PDGF, collagen density, hyaluronan concentration, and fibroblasts. There are many more growth factors and cell types that could be added, but these variables are chosen because they are components of the advanced treatments, and we wish to identify the particular interactions that drive the behavior observed in diabetic wounds. We do not use keratinocytes, even though they do form part of one major treatment (Apligraf™, described below), because they occupy a separate physical location (the epidermis) from the other variables (the dermis). Inclusion of an epidermal compartment is a natural extension of the model. The simulations were run over a time period of 200 days to ensure that a steady state has been reached, i.e., that the wound had stabilized to either a healed or unhealed state.

Within our model, we incorporate a number of wound healing cascade interactions. Monocytes migrate to the wound in response to TGF-β, where they differentiate into macrophages. The phenotype of these macrophages depends on the chemical profile in the wound. The chemical 1,3-β-glucan, which is absorbed into the body from food, encourages inflammatory macrophages to form. Conversely, hyaluronan promotes differentiation into repair macrophages; hyaluronan is produced by fibroblasts within the healing wound. Other macrophage phenotypes may also be present in the wound environment, such as cytocidal macrophages, but as they are not included in the model they will not be discussed here. Macrophages produce growth factors, including TGF-β and PDGF. The former stimulates further macrophage migration into the wound as discussed above. PDGF attracts fibroblasts to the wound, and also induces fibroblast mitosis at low levels. Fibroblasts also produce TGF-β, although not in the same quantities as macrophages. Finally, collagen synthesis by fibroblasts is also induced by TGF-β.

Table 1. Apligraf™ dermal components

Component	100% Density (per mm^3)
Neonatal fibroblasts	500 cells
TGF-β	4 pg
PDGF	1 pg
Collagen	2 μg
Hyaluronan	7.45 μg

TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor.

Modeling these various components of the wound healing process, and their interactions, allows us to investigate details of how the treatments we examine—Apligraf™ and Dermagraft™—actively encourage the diabetic wound to heal, i.e., what "jump starts" the wound into healing?

Apligraf™ (manufactured by Organogenesis, Canton, MA) is an artificial skin comprising a dermal layer and an epidermal layer of cells seeded onto a bioabsorbable scaffold, which can be added to a nonhealing wound.

Based on the average size of a nonhealing diabetic ulcer of $35\,\text{mm}\times25\,\text{mm}\times7.5\,\text{mm}$ ($6{,}562.5\,\text{mm}^3$), the size of one application of Apligraf™ is $1{,}181\,\text{mm}^3$ (assuming there is a 5 mm overlap all the way round the ulcer, as per the treatment instructions). Apligraf™ also has a layer of keratinocytes, but our model focuses on the dermal components of the therapy (Table 1), as discussed above.

The Apligraf™ treatment protocol is one application a week for 5 weeks. Food and Drug Administration (FDA) approval has not been granted for more than five applications to any single wound. Apligraf™ sheets are shipped singly in nutrient medium, in a temperature-controlled container to ensure that the cells are maintained at an incubation temperature of 28 °C during shipping. Owing to this shipping methodology, the cell viability of Apligraf™ is maintained at 100%. More information on Apligraf™ is given in Streit and colleagues.[2,3,18–20]

Dermagraft™ (Smith & Nephew, London, UK) is a dermal substitute comprising fibroblasts seeded onto a Vicryl scaffold. Component densities of Dermagraft™ are given in Table 2.

Based on the average size of a nonhealing diabetic ulcer of $35\,\text{mm}\times25\,\text{mm}\times7.5\,\text{mm}$ ($6{,}562.5\,\text{mm}^3$), the size of one application of Dermagraft™ is $157.5\,\text{mm}^3$ (assuming there is a 5 mm overlap all the way round the ulcer, as per the treatment instructions). The treatment protocol for Dermagraft™ is one application a week for 8 weeks. FDA

Table 2. Dermagraft™ components

Component	100% Density (per mm^3)
Neonatal fibroblasts	8000 cells
TGF-β	0.4 pg
PDGF	1 pg
Collagen	18.75 μg
Hyaluronan	80 μg

TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor.

approval has not been granted for more than eight applications to a single wound.

Dermagraft™ sheets are shipped singly after cryopreservation and require thawing before use. Dermagraft™ may be stored before use provided that it is stored at $-70\,^{\circ}C$. Owing to this shipping methodology, the cell viability of Dermagraft™ is variable. Clinicians do not routinely take samples of the Dermagraft™ before application to the wound; hence the percentage of viable cells being applied is unknown. More detailed information on Dermagraft™ can be found in Naughton and colleagues.[21–24]

It is known that both these treatments can induce healing in chronic diabetic ulcers. Our mathematical model allows us to simulate the behavior of cells, growth factors, and ECM components, and more importantly, to study how they interact. Also, we can investigate how the frequency and strength of the treatment affects the healing wound.

RESULTS

Simulation of treatment protocols

We begin by presenting the results obtained by simulating a typical treatment protocol for each of the treatments.

In a normal wound, the macrophage populations peak first at around days 3–7 and die out, leaving a very low density of macrophages in the wound environment (illustrated in Figure 1). The fibroblast population then peaks at around days 5–10 and eventually returns to the levels of fibroblasts found in undamaged normal skin. Collagen is synthesized by the fibroblasts and reaches the normal undamaged skin density after around 21 days or so from wound inception. Hyaluronan concentration peaks at around the same time as the fibroblasts population and then returns to the undamaged normal skin value. The levels of TGF-β and PDGF within the wound are high initially but quickly reach steady states (Figure 2).

In contrast, in simulations of an untreated diabetic wound, the macrophage populations persist long past the point where they are no longer seen in great numbers in normal wounds, and the inflammatory macrophage population is significantly larger than the repair macrophage population. This increase and persistence in the number of macrophages means that larger amounts of TGF-β and PDGF are observed in the wound. The fibroblast population does not have the same large peak observed in normal wound healing, and the number of fibroblasts within the wound is noticeably lower than in the normal wound. Consequently, collagen accumulation is much lower and even after 200 days there is less collagen in the wound than would be found in normal healed skin. The amount of hyaluronan in the diabetic wound is also significantly lower than in the normal wound, and indeed much lower than that usually found in normal skin. This leads to impaired healing as fewer monocytes differentiate into repair macrophages, and so the macrophage phenotype distribution is disrupted.

Diabetic wounds with healing induced by one of the treatments exhibit similar characteristics to the normal wound healing trajectories. A peak in the fibroblast population occurs, as does a peak in hyaluronan concentration. The macrophage populations reduce in numbers, and the high amounts of growth factors decrease to levels seen in normal wounds. However, although the macrophage population decreases to almost zero, the fibroblast population settles at a density less than that found in normal undamaged skin. The treatment spikes seen in the figures are because the treatment application is modeled as an instantaneous release process rather than as a gradual release process. We make this assumption in view of a lack of data on the effective release rate of the different components into the wound, following treatment applications.

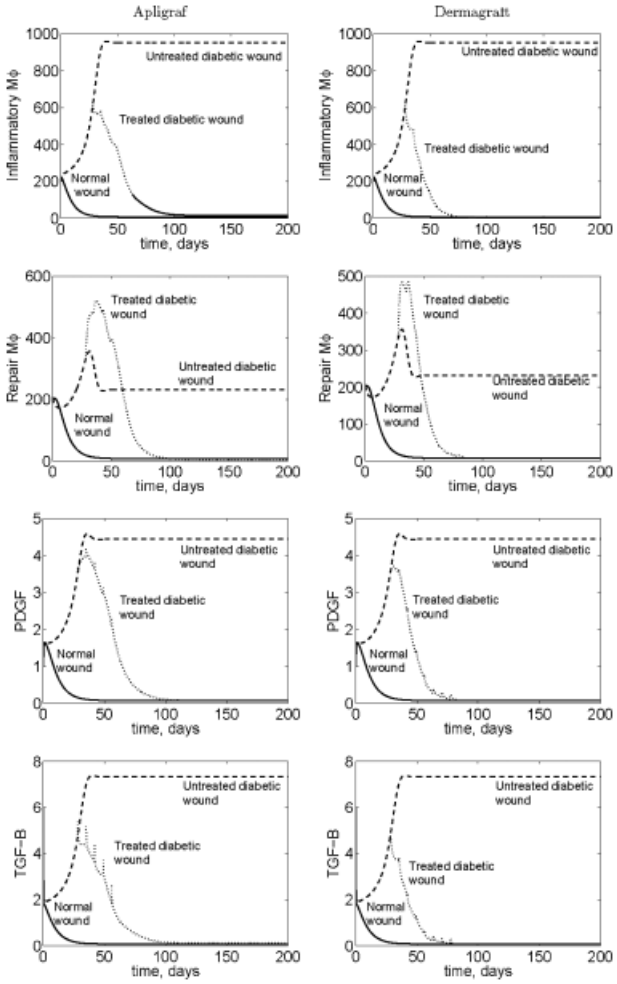

Figure 1. Numerical simulation of our model illustrating normal, untreated diabetic wound and treated diabetic wound healing profiles for inflammatory and repair macrophages, transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF), per cubic mm of wound space, for the Apligraf™ treatment protocol (column I) and Dermagraft™ (column II). The treated diabetic wound simulations show that a healed wound can be obtained within a similar time frame to that found in clinical studies data. Apligraf™ is added to the wound at days 28, 35, 42, 49, and 56, and Dermagraft™ is added to the wound at days 28, 35, 42, 49, 56, 63, 70, and 77, as indicated by the discontinuities in the graphs.

The simulations predict that a course of five applications of Apligraf™ heals the wound within approximately

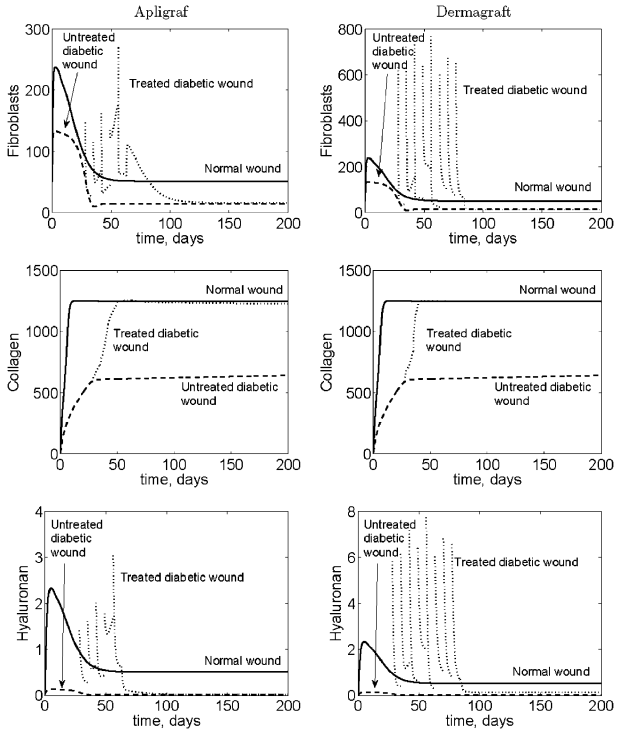

Figure 2. Numerical simulation of our model illustrating normal, untreated diabetic wound and treated diabetic wound healing profiles for fibroblasts, collagen, and hyaluronan, per mm^3 of wound space, for the Apligraf™ treatment protocol (column I) and Dermagraft™ (column II). The treated diabetic wound simulation shows that a healed wound can be obtained within a similar time frame to that found in clinical studies data. Apligraf™ is added to the wound at days 28, 35, 42, 49, and 56, and Dermagraft™ is added to the wound at days 28, 35, 42, 49, 56, 63, 70, and 77, as indicated by the discontinuities in the graphs.

10–11 weeks, and an 8-week course of Dermagraft™ shows wound closure within approximately 9–10 weeks.

These results correlate with healing rates found in available clinical trials data. For patients treated with Dermagraft™, 30% achieved complete healing within 12 weeks, compared with 18% of the control group.[25] Patients treated with Apligraf™ showed a median time to healing of 65 days.[26,27]

Significance of timing and frequency of treatment application

Simulating the application of a single piece of Dermagraft™ to the wound shows that healing can be induced, but that the wound does not appear to be healing for some weeks. Repeating this simulation with a single piece of Apligraf™ shows that this is insufficient to induce wound healing in a diabetic wound.

A complete course of treatments can also be simulated as an equivalent single treatment application. For Dermagraft™, a single treatment equivalent to an 8-week course (i.e., a single application of 8× normal dose) shows that complete wound closure can be achieved within the same time frame as the 8-week course. However, a single treatment of Apligraf™ equivalent to a complete course of five treatments does not promote healing of the wound.

The addition of multiple pieces of Dermagraft™ or Apligraf™ can also be simulated by the model. These simulations indicate that the time to reach complete healing can be reduced by applying two or three pieces of Dermagraft™ each week over the course of the treatment. However, the reduction in healing time is of the order of 5–10 days, which may not justify the increased cost of the complete treatment regime. Similar reductions healing time were predicted using two or three pieces of Apligraf™. Tables 3 and 4 detail the time to healing predicted by the simulations on timing, frequency, and strength of treatment.

Table 3. Results for Dermagraft™

Frequency	Strength	Approximate healing time
Once	×1	140 days
Once	×8	90 days
Once a week for 8 weeks	×1	90 days
Once a week for 8 weeks	×2	85 days
Once a week for 8 weeks	×3	80 days

Simulation of applying individual components of treatments to the wound site

To determine whether the components of these wound healing treatments act alone or synergistically, the model was used to simulate adding individual components to the wound site. We used the standard treatment protocols (one treatment per week over 5 weeks for Apligraf™, and one treatment per week over 8 weeks for Dermagraft™), but simulated the addition of only one of the treatment components, rather than all of them. It was found that adding TGF-β alone to the wound did not encourage healing, nor did wound closure occur when collagen was applied alone. Applying PDGF alone to the wound in amounts normally found in wounds does not induce healing, although healing does occur when very large amounts of PDGF are used. This is the basis for the treatment Regranex™, a gel that contains becaplermin, a recombinant human PDGF, for topical application to the wound. Regranex™ is manufactured by Ortho-McNeil (Titusville, NJ) and is

Table 4. Results for Apligraf™

Frequency	Strength	Approximate healing time
Once	×1	No healing predicted
Once	×8	No healing predicted
Once a week for 5 weeks	×1	100 days
Once a week for 5 weeks	×2	85 days
Once a week for 5 weeks	×3	75 days

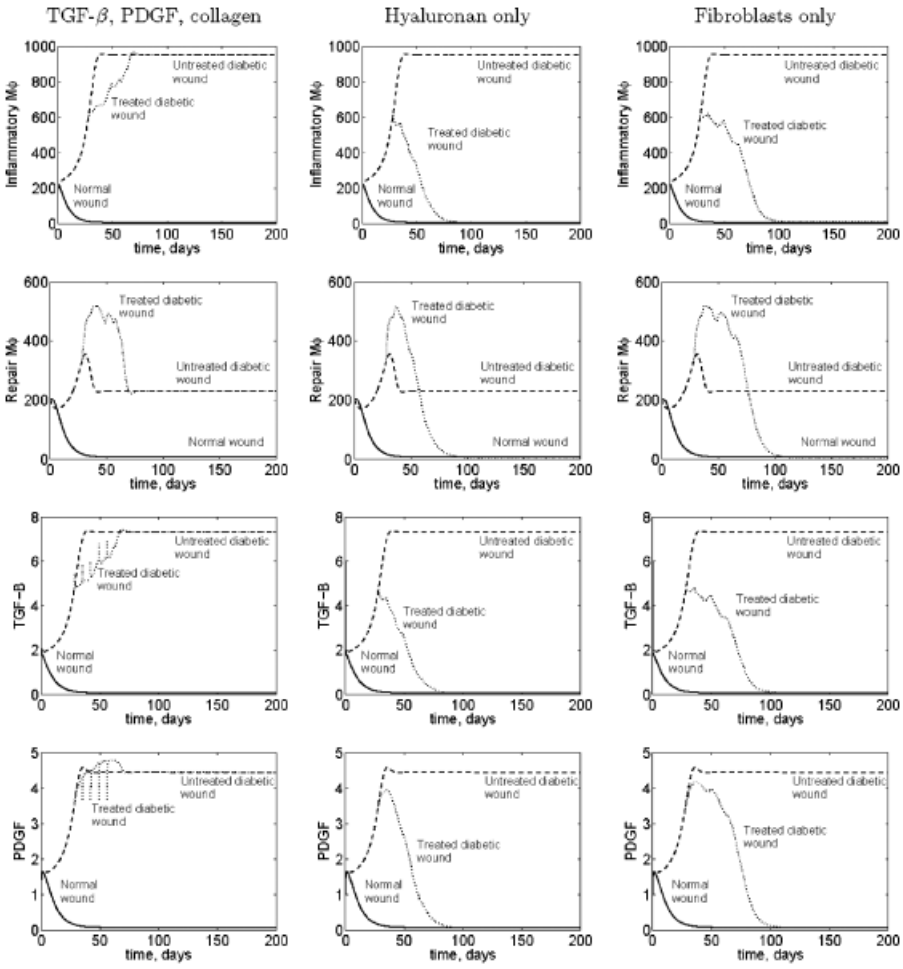

Figure 3. Numerical simulation of our model illustrating normal, untreated diabetic wound, and treated diabetic wound healing profiles for inflammatory macrophages, repair macrophages, transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF), per mm^3 of wound space. The first column shows that adding collagen, TGF-β, and PDGF to the diabetic wound does not induce healing, while the second and third columns show that healing can be induced if either hyaluronan or fibroblasts are added alone. The figures show simulations based on the Dermagraft standard treatment protocol of one treatment per week over 8 weeks. Similar results were obtained based on the Apligraf™ standard treatment protocol of one treatment per week over 5 weeks. TGF-β, PDGF, collagen, Hyaluronan only, and Fibroblasts only.

available in 0.01% strength, i.e., each gram of Regranex™ gel provides 100 μg of becaplermin (recombinant human PDGF-BB). For more information on Regranex™ gel, we direct the reader to Margolis and colleagues.[28–30]

Adding hyaluronan alone in large quantities does encourage wound healing. More hyaluronan means that more monocytes differentiate into repair macrophages and less monocytes become inflammatory macrophages, thus addressing the phenotype imbalance seen in the untreated diabetic wound.

The addition of fibroblasts alone to the wound also induces healing. An increased number of fibroblasts means that hyaluronan synthesis is also increased and this corrects the macrophage phenotype imbalance as described above. To clarify the role of fibroblasts in the treatments, we ran simulations using a hypothetical treatment consisting of modified fibroblasts, which do not synthesize hyaluronan but are otherwise identical to normal fibroblasts. Diabetic wounds treated in this way do not heal, confirming that the key role of fibroblasts in Apligraf™ and Dermagraft™ is as a source of hyaluronan. Simulating the addition of both fibroblasts and hyaluronan to the wound does not give any advantage over the addition of these components individually.

Figures 3 and 4 show that the components do not act synergistically to induce wound healing. The first column shows the effect of adding collagen, TGF-β, and PDGF to the wound, the second shows the effect of adding hyaluronan only, and the third shows the effect of adding fibroblasts only. Based on these results, we predict that only the hyaluronan or fibroblast components of Apligraf™ and Dermagraft are necessary for an effective treatment.

DISCUSSION

There are little data available regarding the measurement of growth factors, ECM components, and cell populations within a healing diabetic wound. One reason for this is that once a diabetic wound appears to be healing, especially if the healing has proved difficult in the past, clinicians may be reluctant to take samples for histological examination and there by risk disrupting the healing process again. Our numerical values for all components can therefore only be classed as best estimates rather than exact. We believe these values to be of the right order of magnitude as correlation with available data for growth factors profiles in acute wounds has been obtained,[31] and the distinctive

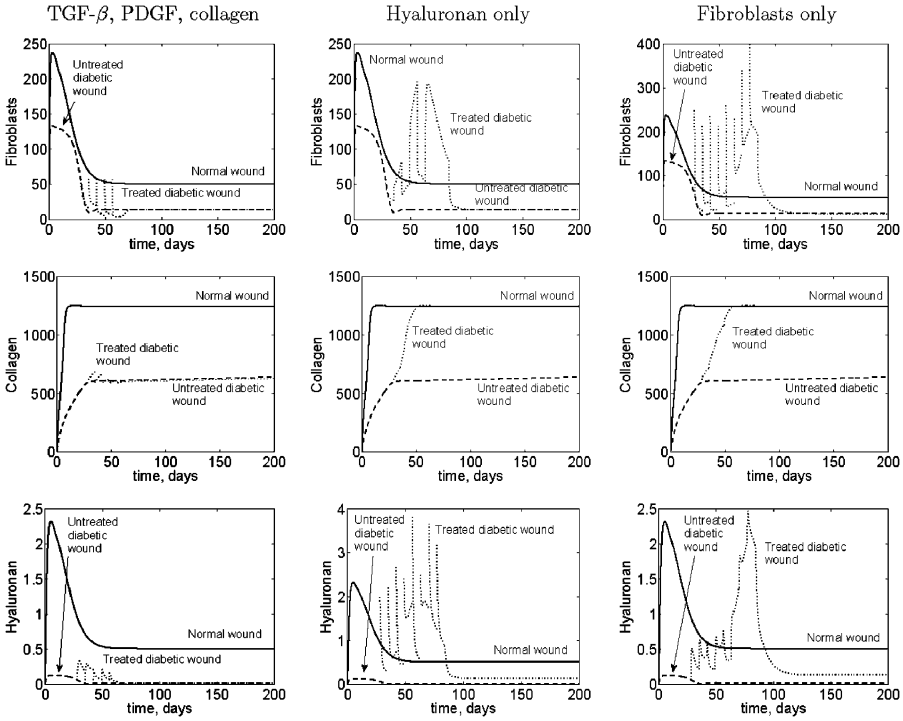

Figure 4. Numerical simulation of our model illustrating normal, untreated diabetic wound and treated diabetic wound healing profiles for fibroblasts, collagen, and hyaluronan, per mm^3 of wound space. The first column shows that adding collagen, transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF) to the diabetic wound does not induce healing, while the second and third columns show that healing can be induced if either hyaluronan or fibroblasts are added alone. The figures show simulations based on the Dermagraft standard treatment protocol of one treatment per week over 8 weeks. Similar results were obtained based on the Apligraf™ standard treatment protocol of one treatment per week over 5 weeks.

peaks in cell populations occur at the appropriate time points as observed by Robson and colleagues.[7,32]

There are obvious limitations with our model. We have only modeled some of the cells, growth factors, and ECM components present in the wound healing process, and have made no distinction between isoforms of collagen, TGF-β, and PDGF. We have deliberately kept the model as simple as possible in order to capture the underlying behavior of diabetic wound healing. We make no attempt to include wound debridement or a bacterial load in the model, and both of these factors are known to influence the successful healing of a diabetic ulcer. Our model also does not make any attempt to include spatial variations of the wound. In practice cell, growth factor and ECM component distribution will be different at the center of the wound to the distribution of components at wound margins, and this may have a significant effect on the repair process.

The model could be extended in a number of directions in future work. A common preparation in diabetic ulcers is debridement, in which damaged and/or otherwise nonviable tissue is removed. This could be simulated in the model by changes to the relevant variables at appropriate time points. However, a lack of data on quantitative changes caused by debridement means that a detailed series of exploratory simulations would be required. Secondly, the model could be used to explore the effects on interpatient variability, by altering parameters such as the growth factor production rates. Again, a detailed and systematic simulation study would be required. Thirdly, the model could be extended to incorporate gradual release of treatment components, rather than the instantaneous release assumed in this paper. This would require the explicit specification of component release as a function of time, and again a further program of simulations would be required, because there are little data available on the effective delivery of the different therapeutic components following treatment application. Finally, the model could be extended to include a separate epidermal component—this is a natural extension of the model as the Apligraf™ treatment protocol involves the addition of keratinocytes as well as dermal components.

From our simulations of adding single components to the wound site, we have seen that wound healing can only be achieved if either fibroblasts or hyaluronan are added to the wound. Wound healing cannot be achieved if small amounts of PDGF are added to the wound, such as those found in normal wounds (about 1–2 pg/mm^3). Interestingly, by adding large amounts of PDGF to the wound, about 650 pg/mm^3, wound healing can be induced. Our simulations indicate that the minimum amount of PDGF needed to encourage wound healing is about 300 pg/mm^3, which is about half the amount found in treatments based on PDGF. Our results suggest that this method works by encouraging large numbers of fibroblasts from beyond the margin of the wound to migrate to the wound site. Once at the wound site, these fibroblasts synthesize hyaluronan and collagen, and the wound starts to heal. A treatment that utilizes this method is Regranex™ (becaplermin gel) described in "Simulation of applying individual components of treatments to the wound site." Based on the average size of a nonhealing diabetic ulcer of 35 mm × 25 mm × 7.5 mm (6,562.5 mm^3), the size of one application of Regranex™ is 385 mm^3 and contains 650 pg PDGF/mm^3. This is far in excess of the levels of PDGF found in normal acute wounds.[31]

Hyaluronan appears to be the key component in the Apligraf™ and Dermagraft™ healing treatments. The simulations show that there may be insufficient hyaluronan in undamaged diabetic skin, in which case a high proportion of monocytes will react to the 1,3-β-glucan stimuli after injury and differentiate into inflammatory macrophages. Dermagraft™ and Apligraf™ add hyaluronan to the wound and this means that more monocytes differentiate into repair macrophages, thus redressing the imbalance between these macrophage phenotypes that has occurred in the diabetic wound.

A recent study by Sheehan et al.[33] using a high molecular weight injection of hyaluronan to treat osteoarthritis of the knee has indicated that hyaluronan may also be involved in disrupting the cell cycle behavior, switching the cells from the $G0/G1$ phases to the S and $G2/M$ phases within 24 hours. This implies that hyaluronan could also be involved in signaling the macrophages to leave the wound site via the lymph node system.[34] This suggests that hyaluronan may have an even more important role in the repair process than that predicted by our model.

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APPENDIX A

In this appendix, we describe the equations of the model and the parameter values used. Each equation gives the rate of change of the variable and its constituent parts. The variables used in the model are inflammatory macrophages (Φ_1, cells/mm^3), repair macrophages (Φ_R, cells/mm^3), TGF-β (T, pg/mm^3), PDGF (P, pg/mm^3), fibroblasts (F, cells/mm^3), collagen (C, μg/mm^3), and hyaluronan (H, μg/mm^3).

Inflammatory macrophages

$$\frac{d\Phi_1}{dt} = \overbrace{\alpha K(T)}^{\text{migration}} + \overbrace{k_1 k_2 \Phi_1 (1 - k_3(\Phi_1 + \Phi_R) - k_5 F - k_6 C)}^{\text{mitosis with crowding effect}} - \overbrace{d_1 \Phi_1}^{\text{death}}.$$

Repair macrophages

$$\frac{d\Phi_R}{dt} = \overbrace{(1-\alpha) K(T)}^{\text{migration}} + \overbrace{k_1 k_2 \Phi_R (1 - k_3(\Phi_1 + \Phi_R) - k_5 F - k_6 C)}^{\text{mitosis with crowding effect}} - \overbrace{d_1 \Phi_R}^{\text{death}}.$$

TGF-β

$$\frac{dT}{dt} = \overbrace{k_4 \Phi_1 + k_7 F}^{\text{production of TGF-}\beta} - \overbrace{d_2 T}^{\text{decay of TGF-}\beta}.$$

PDGF

$$\frac{dP}{dt} = \overbrace{k_8(\Phi_1 + \Phi_R) k_9 F}^{\text{production of PDGF}} - \overbrace{d_3 P}^{\text{decay of PDGF}}.$$

Fibroblasts

$$\frac{dF}{dt} = \overbrace{M(P)}^{\text{migration}} + \overbrace{k_{10} F(1 - k_3(\Phi_1 + \Phi_R) - k_5 F - k_6 C)}^{\text{mitosis with crowding effect}} - \overbrace{d_4 F}^{\text{death}}.$$

Collagen

$$\frac{dC}{dt} = \overbrace{k_{11} F f(T) g(C)}^{\text{production induced by TGF-}\beta\text{ with collagen crowing}} - \overbrace{d_5 F C}^{\text{remodeling}}.$$

Hyaluronan

$$\frac{dH}{dt} = \overbrace{k_{12} F}^{\text{production}} - \overbrace{d_6 H}^{\text{decay}},$$

where α represents the proportion of monocytes migrating to the wound that differentiate into inflammatory macrophages and is represented by $\alpha = -0.197 \log_{10} H + 0.4407$. A logarithmic function was used as a suitable quantitative form for $\alpha(H)$ based on the shape of the curve obtained by plotting the data values for normal skin and Apligraf™ ($H=2.5$, $\alpha=0.5$),[14,35] diabetic skin ($H=0.015$, $\alpha=0.8$),[36] and Dermagraft ($H=80$, $\alpha=0.07$).[37,38]

$K(T)$ is a cubic function of T representing the migration of monocytes to the wound in response to TGF-β (based on data from Waugh and Sherratt[12]), $M(P)$ is a cubic function of P representing the migration of fibroblasts to the wound in response to PDGF (based on data from Facchiano et al.[39]), $f(T)$ is a cubic function of T representing the fibroblast synthesis of collagen in response to TGF-β (based on data from Roberts et al.[17]), and $g(C)$ is a cubic function of C representing the effect increasing collagen density has on its own synthesis by fibroblasts (based on data from Grotendorst et al.[40]). These functions are shown qualitatively in Figure 5.

The parameter values used in the simulations are shown in Table A1. While many of the parameter values were available from previous work or published studies, some of the parameters had to be calculated. In these cases, the source of data used to calculate the parameter values are included, as are sample calculation methods.

Sample parameter calculations are shown below to show how the parameter values are calculated where no published value can be found. For example, the decay rate of PDGF, d_2, is calculated from half-life data, which is around 240 minutes. d_2 can be determined from

$$d_2 = \frac{\ln 2}{T^{1/2}} = \frac{\ln 2}{0.167} = 4 \text{ day}^{-1}.$$

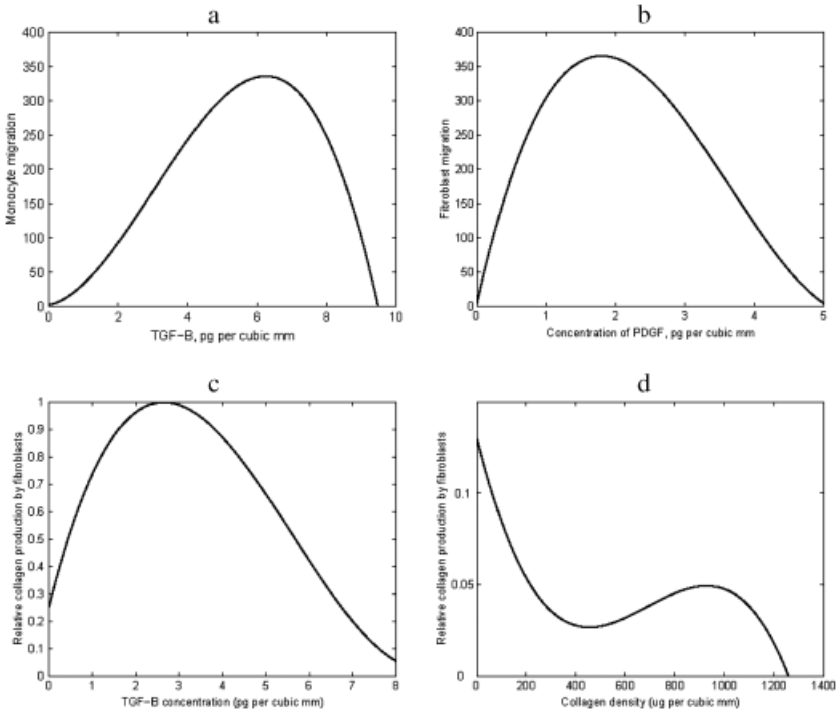

Figure 5. Qualitative form of the functions used in the simulations (A) $K(T)$, which represents monocyte migration in response to transforming growth factor-β (TGF-β), (B) $M(P)$, which represents fibroblast migration to the wound site in response to platelet-derived growth factor (PDGF), (C) $f(T)$, which represents collagen synthesis by fibroblasts in response to TGF-β, and (D) $g(C)$, which represents the effect that collagen density in the wound has on its production by fibroblasts.

Table A1. Model parameter values

Parameter description	Symbol	Value	Units	References
% of macrophages undergoing mitosis	k_1	0.05	—	Miyasaki and colleagues[41,42]
Macrophage growth rate	k_2	0.693	day^{-1}	Calculated[42]
Inverse maximal macrophage density	k_3	0.002	$(cells/mm^3)^{-1}$	Calculated[43]
Macrophage TGF-β production rate	k_4	0.07	pg/cells/day	Cobbold and Sherratt[9]
Inverse maximal fibroblast density	k_5	0.0025	$(cells/mm^3)^{-1}$	Calculated[9]
Inverse maximal collagen density	k_6	0.0004	$(\mu g/mm^3)^{-1}$	Calculated[8,44]
Fibroblast TGF-β production rate	k_7	0.004	pg/cells/day	Huang et al.[45]
Macrophage PDGF production rate	k_8	0.015	pg/cells/day	Badgett et al.[46]
Fibroblast PDGF production rate	k_9	0.0015	pg/cells/day	Baker et al.[31]
Fibroblast growth rate	k_{10}	0.924	day^{-1}	Hehenberger et al.[10]
Fibroblast collagen production rate (diabetic)	k_{11}	5	μg/cells/day	Raghow et al.[16]
Fibroblast collagen production rate (normal)	k_{11}	20	μg/cells/day	Ignotz and Massague[15]
Fibroblast HA synthesis rate (diabetic)	k_{12}	0.001	μg/cells/day	Edward et al.[47]
Fibroblast HA synthesis rate (normal)	k_{12}	0.01	μg/cells/day	Edward et al.[47]
Macrophage removal rate	d_1	0.2	day^{-1}	Calculated[34]
TGF-β decay rate	d_2	9.1	day^{-1}	Cobbold and Sherratt[9]
PDGF decay rate	d_3	4.0	day^{-1}	Calculated[48]
Fibroblast death rate (diabetic)	d_4	2.5	day^{-1}	Darbyvet al.[13]
Fibroblast death rate (normal)	d_4	1.0	day^{-1}	Darbyvet al.[13]
Rate of collagen remodelling by fibroblasts	d_5	1.5×10^{-5}	day^{-1}	Cobbold and Sherratt[9]
Hyaluronan decay rate	d_6	0.7	day^{-1}	Tammi and Tammi[49]

TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor.

The inverse maximal densities are simply the inverse of the maximal density, for example, the maximal fibroblast density is around 400 cells/mm^3 so the the inverse maximal fibroblast density, k_5 is calculated from:

$$k_5 = \frac{1}{400} = 0.0025 \text{ mm}^3/\text{cells}.$$

The growth rate can be determined from population doubling time data. For example, the macrophage growth rate k_2 can be determined from the population doubling time, which is around 1 day. k_2 is therefore

$$k_2 = \frac{\ln 2}{1} = 0.693 \text{ day}^{-1}$$

REFERENCES

1. Marston WA. Dermagraft™, a bioengineered human dermal equivalent for the treatment of the chronic nonhealing diabetic foot ulcer. *Expert Rev Med Devices* 2004; 1: 21–31.
2. Streit M, Braathen LR. Apligraf™—a living human skin equivalent for the treatment of chronic wounds. *Int J Artificial Organs* 2000; 23: 831–3.
3. Leaper D, Paggi B, Compton GA, Orsted H, Teot LM, Ockenfels HM. Growth factors and interactive dressings in wound repair. *EWMA J* 2002; 2: 17–23.
4. Smiell JM, Wieman TJ, Steed DL, Perry BH, Sampson AR, Schwab BH. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower-extremity diabetic ulcer: a combined analysis of four randomised studies. *Wound Repair Regen* 1999; 7: 335–56.
5. Loots A. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. *J Invest Dermatol* 1998; 111: 850–7.
6. Zykova SN. Altered cytokine and nitric oxide secretion in vitro by macrophages from diabetic type II-like db/db mice. *Diabetes* 2000; 49: 1451–8.
7. Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. *Curr Problems Surg* 2001; 38: 65–140.
8. Clark RAF. *The molecular and cellular biology of wound repair*. 2nd ed. New York: Plenum Press, 1996.
9. Cobbold CA, Sherratt JA. Mathematical modelling of nitric oxide activity in wound healing can explain keloid and hypertrophic scarring. *J Theoret Biol* 2000; 204: 257–88.
10. Hehenberger K, Heilborn JD, Brismar K, Hansson A. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal fibroblasts treated with high glucose is associated with increased formation of l-lactate. *Wound Repair Regen* 1998; 6: 135–41.
11. Loots MAM, Lamme EN, Mekkes JR, Bos JD, Middelkoop E. Cultured fibroblasts from chronic diabetic wounds on the lower extremity (non-insulin-dependent diabetes mellitus) show disturbed proliferation. *Arch Dermatol Res* 1999; 291: 93–9.
12. Waugh HV, Sherratt JA. Macrophage dynamics in diabetic wound healing. *Bull Math Biol* 2006; 68: 197–207.
13. Darby A, Bisucci T, Hewitson TD, MacLellan DG. Apoptosis is increased in a model of diabetes-impaired wound healing in genetically diabetic mice. *Int J Biochem Cell Biol* 1997; 29: 191–200.
14. Hascall VC, Laurent TC. Hyaluronan structure and physical properties web document. Available at: http://www.glycoscience.gr.jp, 2000. Accessed 2005.
15. Ignotz RA, Massague J. Transforming growth factor-β stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 1985; 261: 4337–45.
16. Raghow R, Postlethwaite AE, Keski-Oja J, Moses HL, Kang AH. Transforming growth factor-β increases steady state levels of type I procollagen and fibronectin messenger RNAs post-transcriptionally in cultured human dermal fibroblasts. *J Clin Invest* 1987; 79: 1285–8.
17. Roberts AN, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS. Transforming growth factor type β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 1986; 83: 4167–71.
18. Lee KH. Tissue-engineered human living skin substitutes: development and clinical application. *Yonsei Med J* 2000; 41: 774–9.
19. Limova M. New therapeutic options for chronic wounds. *Dermatol Clin* 2002; 20: 357–63.
20. Schmid P. Immunohistologic characterisation of Graftskin (Apligraf™). Wounds: a compendium of clinical research and practice. 2000; 12 (Suppl. A): 4A–11A.
21. Naughton GK. From lab bench to market: critical issues in tissue engineering. *Ann NY Acad Sci* 2002; 961: 372–85.
22. Naughton GK, Mansbridge J, Gentzkow G. A metabolically active human dermal replacement for the treatment of diabetic foot ulcers. *Arti Organs* 1997; 21: 1203–10.
23. Mansbridge J, Lui K, Patch R, Symons K, Pinney E. Three-dimensional fibroblast culture implant for the treatment of diabetic foot ulcers: metabolic activity and therapeutic range. *Tissue Eng* 1998; 4: 403–14.
24. Mansbridge J, Lui K, Pinney E, Patch R, Ratcliffe A, Naughton GK. Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. *Diabetes Obes Metabol* 1999; 1: 265–70.
25. Marston WA, Hanft J, Norwood P, Pollak R, For the Dermagraft Diabetic Foot Ulcer Study Group. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers. *Diabetes Care* 2003; 26: 1701–5.
26. Curran MP, Plosker GL. Bilayered bioengineered skin substitute (Apligraf™): a review of its use in the treatment of venous leg ulcers and diabetic foot ulcers. *BioDrugs* 2002; 16: 439–55.
27. Organogenesis Apligraf™ prescribing information, 2003.
28. Margolis DJ, Crombleholme T, Herlyn M. Clinical protocol: phase I trial to evaluate the safety of H5.020CMV.PDGF-B for the treatment of a diabetic insensate foot ulcer. *Wound Repair Regen* 2000; 8: 480–93.
29. Embil JM, Papp K, Tousignant J, Smiell JM, Wong B, Lau CY. The Canadian becaplermin study group. Recombinant human platelet-derived growth factor-BB (becaplermin) for healing chronic lower extremity diabetic ulcers: an open-label clinical evaluation of efficacy. *Wound Repair Regen* 2000; 8: 162–8.
30. Falanga V. The chronic wound: impaired healing and solutions in the context of wound bed preparation. *Blood Cells Mol Dis* 2004; 32: 88–94.

31. Baker EA, El-Gaddal S, Aitken DG, Leaper DJ. Growth factor profiles in intraperitoneal drainage fluid following colorectal surgery: relationship to wound healing and surgery. *Wound Repair Regen* 2003; 11: 261–7.
32. Singer AJ, Clark RAF. Cutaneous wound healing. *N Engl J Med* 1999; 341: 738–46.
33. Sheehan KM, DeLott LB, Day SM, DeHeer DH. Hyalgan has a dose-dependent differential effect on macrophage proliferation and cell death. *J Othorp Res* 2003; 21: 744–51.
34. Bellingan GJ, Caldwell H, Howie SEM, Branesfield I, Haslett C. In vivo fate of the inflammatory macrophage during resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. *J Immunol* 1996; 157: 2577–85.
35. Kemp P, Bell E, Kagan DT, Mason V, Cavallero J. Preparation of tissue equivalents by contraction of a collagen gel layered on a collagen gel. Patent document US 1996; 5:536,656.
36. Abatangelo G, Martelli M, Vecchia P. Healing of hyaluronic acid-enriched wounds: histological observations. *J Surg Res* 1983; 35: 410–6.
37. Naughton GK, Naughton BA. Three-dimensional cell and tissue culture system. Patent Document US 1990; 4:963,489.
38. Naughton GK, Naughton BA. Three-dimensional skin culture system. Patent document US 1993; 5:266,480.
39. Facchiano A, De Marchis F, Turchetti E, Facchiano F, Guglielmi M, Denaro A, Palumbo R, Scoccianti M, Capogrossi MC. The chemotactic and mitogenic effects of platelet-derived growth factor-BB on rat aorta smooth muscle cells are inhibited by basic fibroblast growth factor. *J Cell Sci* 2000; 113: 2855–63.
40. Grotendorst GR, Martin GR, Pencev D, Sodek J, Harvey AK. Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. *J Clin Invest* 1985; 76: 2323–9.
41. Burke B, Lewis CE, editors. *The macrophage*. 2nd ed. Oxford, UK: Oxford University Press, 2002.
42. Miyasaki K. Chapter 5—Monocytes in basic immunology course notes (web document), UCLA, 2002.
43. Munoz-Alicea R, Negron-Marrero P, Marcano-Velazquez M. *A mathematical model for macrophage, T-cell and mycobacterium tuberculosis actions. Technical report*. Humacao: University of Puerto Rico, 1999.
44. Mast TD. Simulation of ultrasonic pulse propagation through the abdominal wall. *J Acoustical Soc Am* 1997; 102 (Part 1): 1177–90.
45. Huang M, Sharma S, Zhu LX, Keane MP, Luo J, Zhang L, Burdick MD, Lin YQ, Dohadwala M, Gardner B, Batra RK, Strieter RM, Dubinett SM. IL-7 inhibits fibroblast TGF-β production and signalling in pulmonary fibrosis. *J Clin Invest* 2002; 109: 931–7.
46. Badgett A, Bonner JC, Brody AR. Interferon-gamma modulates lung macrophage production of PDGF-BB and fibroblast growth. *J Lipid Mediators Cell Signal* 1996; 13: 89–97.
47. Edward M, Micha D, Tammi RH. Tumour regulation of fibroblast hyaluronan: a mechanism to facilitate tumour growth and invasion. *Carcinogenesis* 2005; 26: 1215–23.
48. Bhandari B, Grandaliano G, Abboud HE. Platelet-derived growth factir (PDGF) BB homodimer regulates PDGF-A and PDGF-B chain gene transcription in human mesangial cells. *Biochem J* 1994; 297: 385–8.
49. Tammi R, Tammi M. Hyaluronan in the epidermis. Glycoscience web forum document. Available at: http://www.glycoscience.gr.jp, 1998. Accessed 2005.