Impossible Experiments using Mathematical Models

Jonathan A. Sherratt

Department of Mathematics
Heriot-Watt University

University of Edinburgh, 24 November 2006
Review of Cutaneous Wound Healing
Scar Formation
TGF-β and Scar Formation
Macrophages and Chronic Diabetic Wounds
New Therapies for Chronic Diabetic Wounds
Using the Model to Understand the Treatments

Collaborators

John Dallon
Steven McDougall
Helen Waugh
Philip Maini
Mark Ferguson

Impossible Experiments using Mathematical Models
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
The basic stages of wound repair

1. Inflammation
2. Proliferation
3. Remodelling
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
Differences between scar tissue and normal dermis include:

- Higher collagen density
- Thicker collagen fibres
- Lower tensile strength
- Different ratios of collagen types
- Different pattern of collagen alignment
Differences between scar tissue and normal dermis include

- Higher collagen density
- Thicker collagen fibres
- Lower tensile strength
- Different ratios of collagen types
- **Different pattern of collagen alignment**: normal tissue has randomly aligned fibres; scar tissue has fibres primarily perpendicular to the basement membrane
Basic Process of Scar Formation

FIBROBLAST REMODELLING
Key Ingredients of a Model

- Fibroblasts tend to move along ECM fibres ("contact guidance")
- New collagen fibres are produced in the direction of cell movement
- Existing collagen fibres are reoriented towards cell movement
We treat cells as discrete objects in a continuum of extracellular matrix.
We treat cells as discrete objects in a continuum of extracellular matrix.

**Fibroblasts:** Represented by a position and direction of movement.
We use realistic cell numbers.
Model Formulation

We treat cells as discrete objects in a continuum of extracellular matrix.

**Fibroblasts:** Represented by a position and direction of movement.
We use realistic cell numbers.

**Extracellular matrix:** Two types: collagen and fibrin.
Mathematically they are represented as vectors with magnitude ↔ ECM density, direction ↔ ECM orientation.
Simulation of Scar Formation
Simulation of Scar Formation
Movies of Wound Healing Simulation

Click here to play the first movie

Click here to play the second movie
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
Experimental work on rats shows that TGF-\(\beta 1\) promotes scar formation and that competitive inhibition of TGF-\(\beta 1\) using mannose-6-phosphate reduces scarring.


This is the basis for the drug Judivex (Renovo PLC): first results from phase II trials due late 2006.
Experimental work on rats shows that TGF-β1 promotes scar formation and that competitive inhibition of TGF-β1 using mannose-6-phosphate reduces scarring.


This is the basis for the drug Judivex (Renovo PLC): first results from phase II trials due late 2006.

Question: what is the mechanism of TGF-β1’s effect on scarring?
A quantitative study of TGF-β1 activity is made possible by the experimental data on concentration vs time in wounds.

(data from Yang et al, Am. J. Pathol. 154:105-111, 1999)
The Effects of TGF-β in Dermal Wound Healing

Standard literature indicates that TGF-β1 controls three important aspects of fibroblast behaviour during dermal wound healing:
The Effects of TGF-β in Dermal Wound Healing

Standard literature indicates that TGF-β1 controls three important aspects of fibroblast behaviour during dermal wound healing:

- Proliferation
Standard literature indicates that TGF-β1 controls three important aspects of fibroblast behaviour during dermal wound healing:

- **Proliferation**
- **Speed**
- **Cell influx**
The Effects of TGF-β in Dermal Wound Healing

Standard literature indicates that TGF-β1 controls three important aspects of fibroblast behaviour during dermal wound healing:

- Proliferation
- Speed
- Cell influx
- Collagen prodn
Unravelling TGF-β1’s Role in Scarring

Normal healing
Unravelling TGF-β1’s Role in Scarring

Normal healing
Unravelling TGF-\(\beta\)1’s Role in Scarring

Normal healing

Removing TGF-\(\beta\)1’s effect on proliferation, motility and collagen prodn
Unravelling TGF-\(\beta1\)’s Role in Scarring

Normal healing

Removing TGF-\(\beta1\)’s effect on proliferation, motility and collagen prodn

Removing TGF-\(\beta1\)’s effect on motility and collagen prodn
Unravelling TGF-β1’s Role in Scarring

- Normal healing
- Removing TGF-β1’s effect on proliferation, motility and collagen production
- Removing TGF-β1’s effect on proliferation and collagen production
Unravelling TGF-β1’s Role in Scarring

Normal healing

Removing TGF-β1’s effect on proliferation, motility and collagen prodn

Removing TGF-β1’s effect on proliferation and motility
Unravelling TGF-$\beta_1$’s Role in Scarring

Normal healing

Removing TGF-$\beta_1$’s effect on proliferation, motility and collagen prodn

More frequent cell reorientation
Unravelling TGF-β1’s Role in Scarring

Normal healing

Removing TGF-β1’s effect on proliferation, motility and collagen prodn

More frequent cell reorientation
Experimental work on rats shows that TGF-β3 reduces scar formation.


This is the basis for the drug Juvista (Renovo PLC): several phase II trials already completed.
Experimental work on rats shows that TGF-β3 reduces scar formation.


This is the basis for the drug Juvista (Renovo PLC): several phase II trials already completed.

Question: what is the mechanism of TGF-β3’s effect on scarring?
TGF-β3 and Scar Formation

Experimental work on rats shows that TGF-β3 reduces scar formation.


This is the basis for the drug Juvista (Renovo PLC): several phase II trials already completed.

**Question:** what is the mechanism of TGF-β3’s effect on scarring?

**Answer:** TGF-β3 increases the frequency of cell reorientation (more filopodia)
TGF-β’s regulate the degree of scarring via their effects on the frequency of fibroblast reorientation.
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
Diabetic Wound Healing

Wound healing in diabetics is impaired
In some cases, wounds fail to heal over long periods (> 6 months ↔ diabetic ulcer)
Limb amputation is required in extreme cases; typical cost in US is $150 (excluding rehabilitation)
Why is Wound Healing Impaired in Diabetics?

- There is not a clear understanding of why wound healing is impaired in diabetics
Why is Wound Healing Impaired in Diabetics?

- There is not a clear understanding of why wound healing is impaired in diabetics
- One component is that some aspects of repair fail to progress beyond the inflammatory phase
Why is Wound Healing Impaired in Diabetics?

- There is not a clear understanding of why wound healing is impaired in diabetics.
- One component is that some aspects of repair fail to progress beyond the inflammatory phase.
- In particular, macrophages persist for long times, with significant numbers present after a month (cf. clearance after a few days in normal healing).
There is not a clear understanding of why wound healing is impaired in diabetics.

One component is that some aspects of repair fail to progress beyond the inflammatory phase.

In particular, macrophages persist for long times, with significant numbers present after a month (cf clearance after a few days in normal healing).

We develop a mathematical model to investigate this persistence of macrophage numbers (and associated phenotype imbalance).
Summary of Key Model Interactions

1,3-B-glucan synthesis

α

Inflammatory macrophages

TGF-β released

Hyaluronic acid synthesis

1-α

Repair macrophages

Monocytes attracted to wound site

Fibroblasts attracted to wound site

Collagen synthesis

PDGF
Model variables: inflammatory macrophages, repair macrophages, fibroblasts, collagen, TGF-β, PDGF, hyaluronan.

Figure 4.6: Results obtained for both normal and diabetic wound healing for the seven equation model. The parameters and function forms used are described in the text.
Apligraf (Organogenesis, US) is an artificial skin comprising dermal and epidermal layers in a bioabsorbable scaffold.

<table>
<thead>
<tr>
<th>Component</th>
<th>100 % Density (per cubic mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Fibroblasts</td>
<td>1550 cells</td>
</tr>
<tr>
<td>TGF-β</td>
<td>0.4 pg</td>
</tr>
<tr>
<td>PDGF</td>
<td>1 pg</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.45 μg</td>
</tr>
<tr>
<td>Total Hyaluronan</td>
<td>18.0 μg</td>
</tr>
<tr>
<td>Dermal Hyaluronan</td>
<td>15.5 μg</td>
</tr>
<tr>
<td>Epidermal Hyaluronan</td>
<td>2.5 μg</td>
</tr>
</tbody>
</table>

Protocol: one application per week for five weeks.
Apligraf (Organogenesis, US) is an artificial skin comprising dermal and epidermal layers in a bioabsorbable scaffold.

<table>
<thead>
<tr>
<th>Component</th>
<th>100 % Density (per cubic mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Fibroblasts</td>
<td>1550 cells</td>
</tr>
<tr>
<td>TGF-β</td>
<td>0.4 pg</td>
</tr>
<tr>
<td>PDGF</td>
<td>1 pg</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.45 μg</td>
</tr>
<tr>
<td>Total Hyaluronan</td>
<td>18.0 μg</td>
</tr>
<tr>
<td>Dermal Hyaluronan</td>
<td>15.5 μg</td>
</tr>
<tr>
<td>Epidermal Hyaluronan</td>
<td>2.5 μg</td>
</tr>
</tbody>
</table>

Protocol: one application per week for five weeks.

A separate modelling study suggests that the epidermal layer does not play a significant role in initiating wound repair.
Dermagraft Treatment

Dermagraft (Smith and Nephew, US) is an artificial dermal layer.

<table>
<thead>
<tr>
<th>Component</th>
<th>100 % Density (per cubic mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Fibroblasts</td>
<td>8000 cells</td>
</tr>
<tr>
<td>TGF-β</td>
<td>0.4 pg</td>
</tr>
<tr>
<td>PDGF</td>
<td>1 pg</td>
</tr>
<tr>
<td>Collagen</td>
<td>18.75 µg</td>
</tr>
<tr>
<td>Total Hyaluronan</td>
<td>80 µg</td>
</tr>
<tr>
<td>Dermal Hyaluronan</td>
<td>80 µg</td>
</tr>
<tr>
<td>Epidermal Hyaluronan</td>
<td>0 µg</td>
</tr>
</tbody>
</table>

Protocol: one application per week for eight weeks.
Simulation of Apilgraff Treatment
Simulation of Dermagraft Treatment
Outline

1. Review of Cutaneous Wound Healing
2. Scar Formation
3. TGF-β and Scar Formation
4. Macrophages and Chronic Diabetic Wounds
5. New Therapies for Chronic Diabetic Wounds
6. Using the Model to Understand the Treatments
Separating the Treatment Components

Included: TGF-$\beta$, PDGF and collagen
Excluded: hyaluronan, fibroblasts
Included: fibroblasts
Excluded: TGF-β, PDGF, collagen and hyaluronan
Fibroblasts secrete a range of regulatory chemicals:

- TGF-\(\beta\)
- PDGF
- collagen
- collagenase
- hyaluronan
Fibroblasts secrete a range of regulatory chemicals:

- TGF-β
- PDGF
- collagen
- collagenase
- hyaluronan

**Question:** which of these are critical to treatment success
Separating Fibroblast Functions

Fibroblasts secrete a range of regulatory chemicals:

- TGF-β
- PDGF
- collagen
- collagenase
- hyaluronan

**Question:** which of these are critical to treatment success

**Answer:** our model predicts that only hyaluronan secretion is critical
Using the model, we simulate treatment with fibroblasts that have been modified to not produce hyaluronan, but are otherwise normal.
Conclusion

- The key component of Apligraf and Dermagraft treatments is the production of hyaluronan by the added fibroblasts.

- Hyaluronan initiates healing by switching macrophages from the inflammatory to the repair phenotype.
Diabetic wound healing can be initiated simply by the addition of hyaluronan.
Review of Cutaneous Wound Healing
Scar Formation
TGF-β and Scar Formation
Macrophages and Chronic Diabetic Wounds
New Therapies for Chronic Diabetic Wounds
Using the Model to Understand the Treatments

1. Review of Cutaneous Wound Healing
   - The basic stages of wound repair

2. Scar Formation
   - Scar vs Normal Dermis
   - Basic Process of Scar Formation
   - Key Ingredients of a Model
   - Model Formulation
   - Simulation of Scar Formation

3. TGF-β and Scar Formation
   - TGF-β1 and Scar Formation
   - TGF-β1 vs Time in Wound Healing
   - The Effects of TGF-β in Dermal Wound Healing
   - Unravelling TGF-β1’s Role in Scarring
   - TGF-β3 and Scar Formation
   - Conclusions

4. Macrophages and Chronic Diabetic Wounds
   - Diabetic Wound Healing
   - Why is Wound Healing Impaired in Diabetics?
   - Summary of Key Model Interactions
   - Typical Model Solution

5. New Therapies for Chronic Diabetic Wounds
   - Apligraf Treatment
   - Dermagraft Treatment
   - Simulation of Apligraf Treatment
   - Simulation of Dermagraft Treatment

6. Using the Model to Understand the Treatments
   - Separating the Treatment Components
   - Separating Fibroblast Functions
   - Demonstration that Hyaluronan Production is Critical
   - Conclusion
   - Prediction