Animal Models of Scarring: The Rabbit Ear Model and its Translational Relevance

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Important Regulators of Scarring

- Genetics (racial characteristics, individual predisposition)
- Impact of prolonged inflammation
- Role of epidermis—Delays in Epithelium: Increased Scar
- Tension
Case 3 – Post-op

Proline sliding
Animal Models

- Relative paucity of models:
  - Loose skinned: no tension
  - Rapid Contraction: rapid healing
- Red Duroc pig
- Rabbit ear model
- Tension model in mice (Gurtner et al)
Hyperpigmentation, hypercontraction, & palpable fibrosis

Epidermal hyperplasia, Excessive melanin production

Minimal differences

Macroscopic appearance

Wound Margin

Unwounded Skin

Yorkshire

Red Duroc

Excessive melanin production
Rabbit Ear Model

- Cartilage Splints Wound: Delayed Epithelization
- Prevents Contraction: so fibroblasts in wound under tension as scar undergoes forces of contraction
Scar Elevation Index Schematic

SEI = \frac{\text{Width of Original Wound} + \text{Hypertrophied Neodermis}}{\text{Cartilage Base}}
Hypertrophic Rabbit Ear Model

- Mimics clinical behavior: aged rabbits have less scarring
- Steroid injections and silicone gel effective
- Clinical and histological appearance similar
- Delayed epithelization: more scarring
- Useful model for testing potential therapeutics
Methods

- Create 7mm punch wounds with removal of perichondrium
- Cover with Tegaderm for 14 days until epithelization is complete
Day 0       Day 7       Day 28            Day 40
Upper row- 5 mm
Lower row- 7 mm
Scatter Elevation Index (Day 28)

p<0.001

Wound Size

Scar Elevation Index, +/- SEM

5mm (n=15)

7mm (n=17)
In hypertrophic scar in rabbit, increase in collagen synthesis correlates to TGFB1 mRNA

No change in TGFB3, TGFB2, or TGFB receptors I, II, or SMAD 3, 4

Kryger, Sisco et al J Am College Surgeons 2007
Results  – *Silicone for 16 days vs. no treatment*

7mm punches, wounds n = 23, grouping per location
Harvest postoperative day 30

**Right Ear**
Silicone POD 14-30 (n = 11) - SEI = 1.14

**Left Ear**
No treatment POD 14-30 (n = 12) - SEI = 1.45

Hypertrophy
70% decrease

*Paired t-test
p < 0.05
Epidermal Thickness Index (ETI)

Ratio of the hypertrophied neoepidermis to the height of the unwounded epidermis \( \Rightarrow \) ETI > 1 = Hypertrophy
Correlation is significant: $r=0.75$, $p<0.0001$

Fig. Correlation of relative epidermal peak thickness and scar peak thickness.
Tape Stripping Day 28

Tape Stripping (2x) 10x

Tape Stripping (2x) 10x
Rabbit Occlusion Model

Scar Elevation Index

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>SEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>1.2</td>
</tr>
<tr>
<td>Tape Stripping</td>
<td>2</td>
</tr>
<tr>
<td>Kelocote</td>
<td>1.4</td>
</tr>
<tr>
<td>Cavilon</td>
<td>1.6</td>
</tr>
<tr>
<td>Indermil</td>
<td>1.8</td>
</tr>
</tbody>
</table>

The graph shows the Scar Elevation Index (SEI) for different treatment groups. Tape Stripping has the highest SEI, indicating a more pronounced scar elevation compared to the other treatments.
Use of Rabbit Ear Model To Explore TGFB Pathways

- Antibody to TGFB: Neutralizing antibody to TGFB1,2,3: Early application—reduction of wound healing. Late application—reduction of scarring
  - *Critical Role of Timing*

- TGFB3: No effect: *Critical Role of Dosing*

- Antisense to TGFB, CTGF
CTGF appears to mediate some of the pro-fibrotic and proliferative effects of TGF-β

- Potentiates sustained fibrosis when injected with TGF-β
- TGF-β induces the CTGF promoter via PKC/Ras/MEK/ERK and via Smad3
- CTGF response element exists on the Col I promoter
Introduction

- Antisense oligonucleotides have shown promise as a therapeutic modality in oncology, inflammatory, and viral infective disease.
Hypotheses

- CTGF expression is sustained at high levels in hypertrophic scars
- Early OGN blockade of CTGF will not affect scarring or wound healing
- Late OGN blockade of CTGF will abrogate scarring
Results

CTGF expression is sustained at high levels in hypertrophic scars
Results

Late Injection of Anti-CTGF OGNs reduce SEI by 43%

![Bar chart showing the effect of different treatments on Scar Elevation Index (SEI). The chart compares No treatment, Scramble OGN (n=30), and Anti-CTGF OGN (n=25). The Anti-CTGF OGN group shows a significant reduction in SEI compared to the other groups, p<0.001.]
Effects of Adipose tissue-derived Adult Progenitor Cells on Hypertrophic Scar Formation in the Rabbit Model

Theodore W. Nagel, Oliver Kloeters, SX Jia, Xianzhong Ding, Thomas A. Mustoe, John Y.S. Kim

Wound Healing Research Laboratory, Division of Plastic Surgery
Northwestern University Feinberg School of Medicine
Methods: Cell Isolation

Results: Scar Elevation Index

Scar Elevation Index

Control

0
1
2
3

p=0.001
p=0.058
p=0.73
Results: Inflammatory Cells

Inflammatory Cells

# cells / Area

p=0.008
p=0.2
p=0.21
Conclusions

- The rabbit ear model has many clinically relevant features
  - Delayed epithelization resulting in prolonged inflammation
  - Tension secondary to splinting effect
- For current therapeutic maneuvers, the model behaves like human wounds
- New therapeutic strategies can be tested