Syndesome-Based Dressings for Enhanced Wound Healing in Diabetic Ulcers

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UNLABELED/UNAPPROVED USES DISCLOSURE:
None.
Wounds – The Clinical Problem

- $50B spent per year – wound care in USA
- 6.5 million chronic wound patients
- Increasing prevalence of PVD and Diabetes with an ageing population

- Diabetes – 347 million patients (2011)
- PVD – 202 million patients (2010)

Global Wound Care Market (2013)

Growth Factors
3.4%

- Bioengineered Skin: 2.9%
- Negative Pressure Wound Therapy: 14.5%
- Antimicrobial Dressings: 11%
- Hydro-colloids: 6.5%
- Foam Dressings: 7.9%
- Traditional Adhesive Bandages: 26.8%
- Traditional Gauze Bandages: 10.5%
- Non-Adherent Bandages: 8.3%
- Film Dressings: 3.5%
An appealing strategy for treating cutaneous wounds is to use growth factor proteins or genes to stimulate the native tissue to repair itself.
Growth Factor Therapy Trials

- Topical FGF-2 for healing diabetic foot ulcers – no benefit over placebo. ¹
- Topical EGF for venous ulcers – non significant effects. ²
- Becaplermin (PDGF-BB) or Regranex™ gel – only growth factor approved by the Food and Drug Administration (FDA) for neuropathic diabetic foot ulcers. ³

Overall Motivation

**Problem**
Negligible long-term results in human clinical trials

**Appealing Strategy**
Growth factor proteins or genes

**Non-healing Wounds**
50 billion $’s spent

**Overall Goal**
1. To overcome resistance to growth factor therapy
2. To test therapeutic in a clinically relevant mouse model
Ob/Ob Mouse Model

- Deficient for hormone Leptin
- Characteristics:
  - Obesity
  - Hyperphagia
  - Hyperglycemia
  - Glucose Intolerance
  - Insulin Resistance
- High fat diet for 10 weeks
Does Ob/Ob Mouse Exhibit Resistance to Growth Factor Therapy?
Ob/Ob Mice Exhibit Growth Factor Resistance

Growth Factor Interactions

INITIATION of angiogenesis
- Detachment of pericytes
- Degradation of basement membrane

Hif1-a
VEGF
Ang-2

NEOVESSEL FORMATION
- Endothelial cell proliferation and migration
- Pericyte proliferation

VEGFs
FGFs

ADAPTATION to tissue needs
- Regression of neovessels due to lack of flow or presence of growth factors

Ang-2

MATURATION
- Attachment of pericytes
- Deposition of basement membrane

PDGFs
Ang-1

Growth Factor

Co-receptor
Receptor

Co-receptor
Syndecan-4

Syndecan-4 in Mouse Tissues

**MUSCLE**

- **HFD:** High Fat Diet (10 weeks)
- **NCD:** Normal Chow Diet (10 weeks)

**HEART**

- **HFD:** High Fat Diet (10 weeks)

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**Norm. Protein Expression**

**Strain:**
- WT
- Ob/Ob

**Diet:**
- NCD
- HFD

*p < 0.05*

Reduction of Syndecan-4 in Human Tissue

Non-Diabetic

Diabetic
Reduction of Syndecan-4 in Human Tissue

![Image of tissue sections for Non-Diabetic and Diabetic groups]

- **Non-Diabetic**
  - Image of tissue section showing Syndecan-4 expression

- **Diabetic**
  - Image of tissue section showing reduced Syndecan-4 expression

![Bar chart showing Syndecan-4 Positive Cells (%)]

- **Y-axis:** Syndecan-4 Positive Cells (%)
- **X-axis:** Non-Diabetic / Diabetic

- Bar height indicates higher expression in Non-Diabetic compared to Diabetic samples.

*Statistical significance indicated by asterisk:*
Growth Factor Resistance

Syndecan-4

FGFR-1

FGF-2

Syndecan-4
Can We Overcome This Resistance to Growth Factor Therapy?

Syndesomes will be referred as **S4PL** from here on.
Characterization of Syndesomes

![Graph showing size distribution of S4PL particles](image)

- Intensity (%) vs. Size (nm)
- S4PL

![Electron microscopy image](image)

Scale: 400 nm
S4PL+FGF-2 Enhance Angiogenesis in a Subcutaneous Implantation

S4PL+FGF-2 Enhance Angiogenesis in Ischemic Tissues

Day 0

Day 7

Day 14

Poster Number 493
Can Syndesomes Enhance Wound Healing?

Syndesomes

Syndesome/Alginate Wound Dressing

Improved Wound Healing in Chronic Wounds
ECIS - Electric Cell-substrate Impedance Sensing
S4PL Enhances *In Vitro* Migration

**NORMAL KERATINOCYTES**

**DIABETIC KERATINOCYTES**
S4PL Enhances *In Vitro* Migration

**NORMAL FIBROBLASTS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Δ Resistance (k-ohm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Treatment</td>
<td>0</td>
</tr>
<tr>
<td>FGF-2</td>
<td>0.15</td>
</tr>
<tr>
<td>1:1000 S4PL</td>
<td>0.1</td>
</tr>
<tr>
<td>1:500 S4PL</td>
<td>0.05</td>
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<tr>
<td>1:250 S4PL + FGF-2</td>
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**DIABETIC FIBROBLASTS**

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4% Alginate Disk Preparation
Mouse Excisional Wound Model Procedure
S4PL + FGF-2 Enhance Wound Closure
S4PL + FGF-2 Enhances Wound Closure

- **Control**
- **S4PL**
- **FGF-2**
- **S4PL + FGF-2**

**Open Wound Area (%)**

- **Day 0**
- **Day 7**
- **Day 14**

*Note: The diagram shows the percentage of open wound area over time for different treatment groups. The bars represent the mean ± standard error. The asterisk (*) indicates a statistically significant difference between groups at Day 14.*
Movat’s Pentachrome Staining of the Wound Sections
S4PL + FGF-2 Increases Epidermis Length

W: Wound bed  F: Fat layer
S4PL + FGF-2 Increases Epidermis Length

* significant w.r.t. all other groups. p<0.05
S4PL + FGF-2 Enhance Wound Perfusion
S4PL + FGF-2 Increases Vasculature in the Wound Bed

* significant w.r.t. all groups, #significant w.r.t control and S4PL groups. p<0.05
S4PL Decreases CD86^+ Macrophages

* significant w.r.t. FGF. p<0.05
S4PL Increases CD163⁺ Macrophages

* significant w.r.t. FGF. p<0.05
S4PL + FGF-2 Reduces Macrophage Count in the Wound
S4PL + FGF-2 Increases CD206⁺ M2 Macrophages
S4PL + FGF-2 Enhances IL-4 Expression at Day 6
S4PL Enhances IL-6 Expression at Day 2

[Graph showing IL-6 concentration (pg/ml/mg of tissue) over Days 2 and 6 for different conditions: Control, FGF-2, S4PL, S4PL + FGF-2.]
S4PL+FGF-2 also Increases other Cytokines at Day 6
Summary

Day 14

- FGF-2
- VEGF-A
- PDGF-

S4PL + FGF-2

FGF-2

S4PL

Alginate

Day 0

- Control
- Ischemic

Day 14

- Control
- FGF-2
- S4PL
- S4PL + FGF-2

CD163 Positive Cells (%)

Control

FGF-2

S4PL

S4PL + FGF-2

*
Acknowledgements

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Contact: subhamoy.das@utexas.edu
S4PL Enhances *In Vitro* Migration

**NORMAL KERATINOCYTES**

**DIABETIC KERATINOCYTES**
Excisional Wound Model

HUMANS
- Wound heals by formation of granulation tissue and re-epithelialization.
- Skin is tethered to subcutaneous tissues, hence no contraction.

RODENTS
- Wound heals primarily by contraction and some regeneration.
- Skin is mobile, hence more contraction.

In PAD patients and diabetics, the process of wound healing is substantially impaired, leading to chronic wounds.
