3-26-2014

Physiology and Advancements in Wound Healing

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Physiology and Advancements in Wound Healing

Adam L. Baker, MD PGY-4

Advisor:
Edmund Pribitkin, MD
Outline

• Fundamentals
• Advances
  – Growth factors
  – Platelet Rich Plasma
  – Engineered skin
  – Hyperbaric Oxygen Therapy
• Research
Archduke Franz Ferdinand
The Great War
Alexis Carrel, MD

Henry Drysdale Dakin, PhD
Carrel Apparatus
Carrel-Dakin solution

APPARATUS FOR APPLYING CARREL-DAKIN SOLUTION

This apparatus is furnished by instrument dealers, supplied by Johnson & Johnson on request.

A—Reservoir graduated.
B—Clamp for regulating flow.
C—Sight feed cup.
D—Four-way glass distributor.
E—Perforated distributing tubes with ends tied. When used for surface ends are covered with Turkish toweling.

F—Five-way glass distributor.
G—One tube glass distributor.
H—Two-way glass distributor.
I—Syringe for applying solution by hand.
J—Flask for use with syringe.
Use of Carrel Apparatus
Alexis Carrel, MD

Henry Drysdale Dakin, PhD
Skin Anatomy

- Epidermis
  - Corneum
  - Lucidum
  - Granulosum
  - Spinosum
  - Basale
- Dermis
- Hypodermis
Pilosebaceous Unit

- Consists of:
  - hair follicle
  - sebaceous gland
  - eccrine gland
  - apocrine gland
Wound Healing

- Phases
  - Hemostasis
  - Inflammation
  - Proliferation
  - Maturation
  - Remodeling
Wound Healing

Hemostasis:
platelet activation

Cellular influx

Inflammation

Inflammation

Days Postwounding

Days Postwounding

Relative Number of Cells

Relative Number of Cells

Neutrophils

Macrophages

Fibroblasts

Lymphocytes

Hematogenesis

Proliferation

Maturation

Re-epithelialization

Keratinocytes

Type III collagen now type I

Fibroblast

Apoptosis vs. Synthesis balance

Excessive fibrosis lead to scar

Fibroblasts
Wound Healing

- Inflammation
- Proliferation
- Maturation

Graph showing:
- Relative Amounts of Matrix Synthesis
- Fibronectin
- Collagen III
- Collagen I

Days Postwounding:
- 0
- 2
- 4
- 6
- 8
- 10
- 12
- 14
- 16

Wound breaking strength
Macrophage: The QB
## Wound Healing: Growth Factors

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Biologic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet-derived growth factor (PDGF)</td>
<td>Proliferation, chemotaxis, matrix synthesis</td>
</tr>
<tr>
<td>Transforming growth factor- (TGF)</td>
<td>Inflammation, granulation</td>
</tr>
<tr>
<td>Vascular endothelial growth factor (VEGF)</td>
<td>Angiogenesis</td>
</tr>
<tr>
<td>Fibroblast growth factor (FGF)</td>
<td>Granulation, re-epith</td>
</tr>
<tr>
<td>Keratinocyte growth factor (KGF)</td>
<td>Re-epithelialization</td>
</tr>
</tbody>
</table>
Wound Healing: Growth Factors

Inflammatory Phase (Day 3)

Fibrin clot

Macrophage

TGF-β1

TGF-α

FGF

VEGF

PDGF BB

IGF

KGF

PDGF

TGF-β1

TGF-β2

TGF-β3

Fibroblast

Fat

Dermis

Blood vessel

Platelet plug

Neutrophil

VEGF

FGF-2

Wound Healing: Growth Factors

Epidermis
A soldier returns from Ypres with a major facial injury.

Cartilage is implanted in the forehead and left to heal.

Retaining the blood supply, the cartilage is twisted into position.

Once healed, the excess tissue at the top of the nose is removed.

In his sixties, the patient’s scars are barely visible.
Advances in Wound Healing: Exogenous Growth Factors

• PDGF (Regranex) approved in 1998 by FDA for use in diabetic foot ulcers
  – EBM I, 48% vs 25%23

• FGF
  – Venous ulcers, diabetic wounds24
    • Inconsistent results
    – Tympanic Membrane perforations

• KGF
  – Mucositis

• VEGF
  – Diabetic Ulcers


Regenerative Treatment for Tympanic Membrane Perforation

*†Shin-Ichi Kanemaru, ‡Hiroo Umeda, †Yoshiharu Kitani, §Tatsuo Nakamura, †Shigeru Hirano, and †Juichi Ito

Trafermin (Fifbrast) Recombinant Human basic Fibroblast Growth Factor (b-FGF)
Regenerative Treatment for Tympanic Membrane Perforation

1. TM perforation
2. Disruption of the perforation edge
3. Gelatin sponge with b-FGF
4. Fibrin Glue
5. After 3 weeks
Regenerative Treatment for Tympanic Membrane Perforation
Regenerative Treatment for Tympanic Membrane Perforation

N = 56
63 TMP

 TMP s/p OM inflammation
Old traumatic TMP
TMP s/p tube

53 bFGF

10 gelfilm

Outcomes measures
• Closure
• Hearing Level
• Sx Sequela
Regenerative Treatment for Tympanic Membrane Perforation
Regenerative Treatment for Tympanic Membrane Perforation

Results
Regenerative Treatment for Tympanic Membrane Perforation

Results
Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers


Recombinant Human Keratinocyte Growth Factor (rhKGF)
Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers

Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers


Intensive Therapy
- Whole body irradiation
- Chemotherapy
- Bone Marrow Transplantation

N = 212

3x IV Palifermin

Hodkin’s Disease
Non-Hodgkin’s Lymphoma
Leukemia
Multiple Myeloma

3x IV Placebo

3x additional doses after BMT
<table>
<thead>
<tr>
<th>Variable</th>
<th>Palifermin Group (N=106)</th>
<th>Placebo Group (N=106)</th>
<th>P Value*</th>
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</thead>
<tbody>
<tr>
<td><strong>Oral mucositis of WHO grade 3 or 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence --- no. of patients (%)†</td>
<td>67 (63)</td>
<td>104 (98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Duration --- days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–22</td>
<td>0–27</td>
<td></td>
</tr>
<tr>
<td>Patients with oral mucositis of WHO grade 3 or 4</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>6.0</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1–22</td>
<td>1–27</td>
<td></td>
</tr>
<tr>
<td><strong>Patient-reported outcomes (AUC)‡</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score for soreness of mouth and throat</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>29.0</td>
<td>46.8</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0–98</td>
<td>0–110</td>
<td></td>
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<tr>
<td>Swallowing-limitation score</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>Median</td>
<td>22.5</td>
<td>38.3</td>
<td></td>
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<tr>
<td>Range</td>
<td>0–104</td>
<td>0–104</td>
<td></td>
</tr>
<tr>
<td><strong>Functional Assessment of Cancer Therapy general score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical well-being domain</td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Median</td>
<td>736.6</td>
<td>712.1</td>
<td></td>
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<tr>
<td>Range</td>
<td>176–1033</td>
<td>176–1014</td>
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<tr>
<td>Functional well-being domain</td>
<td></td>
<td></td>
<td>0.036</td>
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<tr>
<td>Median</td>
<td>546.1</td>
<td>542.5</td>
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<tr>
<td>Range</td>
<td>93–985</td>
<td>93–1043</td>
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</tr>
</tbody>
</table>
Topical Vascular Endothelial Growth Factor Accelerates Diabetic Wound Healing through Increased Angiogenesis and by Mobilizing and Recruiting Bone Marrow-Derived Cells

Robert D. Galiano,* Oren M. Tepper,* Catherine R. Pelo,* Kirit A. Bhatt,* Matthew Callaghan,* Nicholas Bastidas,* Stuart Bunting,† Hope G. Steinmetz,† and Geoffrey C. Gurtner*

Average healing time
12 days vs. 25 days
(VEGF vs control)

Systemic absorption:
18 days vs. 25 days
(PBS vs control)
Growth Factors: Limitations

• Cost
  — Regranex $586 per 15g tube
• Delivery
  — Exception
• Risk of Malignancy
  — 2008 retrospective study
• Lack of data!
Marie Curie: Portable X-ray

Renault truck outfitted with mobile x-ray equipment
Platelet Rich Plasma (PRP)
Platelet Rich Plasma (PRP)

Platelet-derived growth factor (PDGF)
Epidermal Growth Factor (EGF)
Transforming Growth Factor beta (TGF-β)
Vascular Endothelial Growth Factor (VEGF)
Fibroblast Growth Factor (bFGF)
Epidermal Growth Factor (EGF)

CaCl$_2$
Thrombin
The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds

David B. Hom, MD; Bradley M. Linzie, MD; Trevor C. Huang, PhD
The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds

David B. Hom, MD; Bradley M. Linzie, MD; Trevor C. Huang, PhD

Table 3. Growth Factor Assays (ELISA)*

<table>
<thead>
<tr>
<th>Growth Factors</th>
<th>Initial Blood Sample (60 mL)</th>
<th>PRP (6 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGF-AB, ng/mL</td>
<td>10.2 ± 1.4</td>
<td>88.4 ± 28.8</td>
</tr>
<tr>
<td>PDGF-AA, ng/mL</td>
<td>2.7 ± 0.5</td>
<td>22.2 ± 4.2</td>
</tr>
<tr>
<td>PDGF-BB, ng/mL</td>
<td>5.8 ± 1.4</td>
<td>57.8 ± 36.6</td>
</tr>
<tr>
<td>TGF-β1, ng/mL</td>
<td>41.8 ± 9.5</td>
<td>231.6 ± 49.1</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>83.1 ± 65.5</td>
<td>597.4 ± 431.4</td>
</tr>
<tr>
<td>bFGF, pg/mL</td>
<td>10.7 ± 2.9</td>
<td>48.4 ± 25.0</td>
</tr>
<tr>
<td>EGF, pg/mL</td>
<td>12.9 ± 6.2</td>
<td>163.3 ± 49.4</td>
</tr>
</tbody>
</table>
The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds

David B. Hom, MD; Bradley M. Linzie, MD; Trevor C. Huang, PhD

Control Site

APG-Treated Site

Day 0
A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

Jamal M. Bullocks · Anthony Echo · Gerardo Guerra · Samuel Stal · Eser Yuksel
A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

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A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

Jamal M. Bullocks · Anthony Echo · Gerardo Guerra · Samuel Stal · Eser Yuksel

Diced cartilage
A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

Jamal M. Bullocks · Anthony Echo · Gerardo Guerra · Samuel Stal · Eser Yuksel
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A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

Jamal M. Bullocks · Anthony Echo · Gerardo Guerra · Samuel Stal · Eser Yuksel

N = 68
2005-2008

PRP-Scaffold Rhinoplasty

Mean follow up 15 months

Complications
• 11 transient erythema
• No resorption during f/u

No explantation of graft
PRP in Tympanic Membrane Perforations

• Evidence in Rats
  – Accelerates
• Case reports in humans with some success
PRP: jury’s still out....

- Cochrane review 2012,
  - No difference in tx chronic wounds
  - “poor design of previous trials”
PRP
FACIAL REJUVENATION

Revitalize Skin - Eliminate Wrinkles and Dark Circles - Heal Damage

Platelet Rich Plasma (PRP)

Platelet rich plasma is a 100% natural method that uses the patient's own blood components to stimulate the renewal of damaged tissue in areas of the face, neck, decollete, hands and body.

This four-step procedure is found to have great efficacy in revitalizing skin and eliminating wrinkles, sagging and dark circles, while healing skin damaged by acne, injuries and stretch marks.

Extracted blood platelets that contain proteins, nutrients, and a variety of growth factors are injected into the site of concern. When growth factors are activated by injection, repair of damaged tissue begins, generating collagen and hyaluronic production.

A natural enhancement of the skin's appearance begins to take place and youthfulness, suppleness and volume are restored to the treated areas.
# Bioengineered skin

<table>
<thead>
<tr>
<th>Tissue Material</th>
<th>Tissue Layers</th>
<th>Living</th>
<th>Trade Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured keratinocytes autograft</td>
<td>Epidermal</td>
<td>Yes</td>
<td><a href="#">Epicel</a> (cultured epidermal autografts)</td>
</tr>
<tr>
<td>Acellular free-dried cadaveric skin allograft</td>
<td>Dermal</td>
<td>No</td>
<td><a href="#">AlloDerm</a></td>
</tr>
<tr>
<td>Bovine collagen/glycosaminoglycan/Silastic</td>
<td>Dermal</td>
<td>No</td>
<td><a href="#">INTEGRA</a></td>
</tr>
<tr>
<td>Neonatal</td>
<td>Dermal</td>
<td>Yes</td>
<td><a href="#">Dermagraft</a></td>
</tr>
<tr>
<td>Neonatal fibroblasts/polyglactin mesh allograft</td>
<td></td>
<td></td>
<td><a href="#">Apligraf</a></td>
</tr>
<tr>
<td>Neonatal fibroblasts/keratinocytes collagen allograft</td>
<td>Composite</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>
Bioengineered skin: Apligraf

• In vitro construct of human skin

**Neonatal foreskin**

- Epidermal keratinocytes
- Dermal fibroblasts with a matrix of type I collagen

Perform serial passage and culture

**Dermis:** fibroblasts in collagen deposit,

**Epidermis:** cultured keratinocytes on top of the dermis


**Development of a bilayered living skin construct for clinical applications.**

*Wilkins LM*, Watson SR, Prosky SJ, Meunier SF, Parenteau NL.*
Bioengineered skin: Apligraf
Hyperbaric Oxygen
Hyperbaric Oxygen

1 atm

55 mm Hg

2000 mm Hg

3 atm

500 mm Hg

2000 mm Hg


Hyperbaric oxygen: its mechanisms and efficacy.
Thorn SR
Hyperbaric Oxygen

HBO₂ → Elevated Cellular O₂ Levels → Increased ROS & RNS

- Increase wound growth factors synthesis
- SPCs mobilization from bone marrow
- Neutrophil β-actin S-nitrosylation
- Lower monocyte chemokine synthesis
- Ischemic preconditioning changes in HO-1, HSPs, HIF-1

- Elevated tissue: SDF-1, Angiopontin, Basic fibroblast GF, transforming GF β1, VEGF (via HIF-1)
- Increased peripheral site SPCs HIF-1/2 content & HIF-related gene products
- Impaired β₂ integrin function

- Diminished inflammatory responses

- Improved neovascularization
- Improved post-ischemic tissue survival

Hyperbaric oxygen: its mechanisms and efficacy.
Thom SR
Hyperbaric oxygen therapy for late radiation tissue injury

Michael H Bennett¹, John Feldmeier², Neil Hampson³, Robert Smee⁴, Christopher Milross⁵

11 RCT, N = 669

2001-2011

Significant Results:

1.) Primary Tx of ORN
2.) Following Surgical excision
3.) Healing irradiated tooth sockets following dental extraction
An Evidence-Based Appraisal of the Use of Hyperbaric Oxygen on Flaps and Grafts

H. I. F. Friedman, M.D., Ph.D.
M. Fitzmaurice, M.D.
J. F. Lefavre, M.D.
T. Vecchiolla, M.S.N.
D. Clarke

Columbia, S.C.

• Animal studies
  – Decreased distal necrosis
  – Free flaps, allowed prolonged ischemia

• Clinical studies
  – Cochrane review: 1 RCT STSG
  – “...high risk of bias”
  – “…more data needed”
Research

• Purpose: Characterize histologic and biochemical effects of age and exercise on axial based flaps.

• Plan: Develop an animal model
  – Fasiculocutaneous flaps in Sprague Dawley rats
  – Perturb the model: age and exercise
Design

- Flap
- Pedicle
- Defect
- 3 cm
- 8 cm
Old vs. Young

B.

Percent of necrosis (%)
Increasing Akt activation
Initial Conclusions  What this really means....

- Young vs. old
  - Increased VEGF
  - Increased Atk
  - Decreased apoptosis

- Flaps do better
  - More vascular
  - Heal faster
  - Less necrosis
Exercise?

- 2 weeks of exercise prior to flap harvest
- 4 groups
  - Old, Young +/- exercise
Effect of Exercise: Young Rats

Flap area

IB:
- p-Akt (S473)
- Akt (pan)
- p-Akt (S473)
- Akt (pan)

Time post-surgery:
0 | 2 days | 5 days | 9 days

Young Rats (YR) vs. Young Endurance Rats (YE)
Exercise Old Rats

Flap area

<table>
<thead>
<tr>
<th>IB:</th>
<th>p-Akt (S473)</th>
<th>Akt (pan)</th>
<th>p-Akt (S473)</th>
<th>Akt (pan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OE</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Time post-surgery

<table>
<thead>
<tr>
<th>0</th>
<th>2 days</th>
<th>5 days</th>
<th>9 days</th>
</tr>
</thead>
</table>
Conclusions

• Cardiovascular exercise
  ─ Increase in VEGF in both exercising groups
  • Old exercising group higher response % increase in VEGF
  ─ Increase in Atk in both exercising groups
Next Steps?

• Other markers of wound healing
  – bFGF
  – EGF
  – PDGF
• Effects of alcohol
Gratitude

• Department Otolaryngology, Dr. Keane
• Dr. Pribitkin
• Rat flap team
  – Sudeep Roy MD
  – Beth Duddy
  – Salini Hota, Li-Hui Zhang
  – Dr. Edita Aksamitiene
  – Dr. Joannes Hoek