3-26-2014

Physiology and Advancements in Wound Healing

Adam Baker, MD
Thomas Jefferson University

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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
Carrel Apparatus
Carrel-Dakin's 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.
J—Syringe for applying solution by hand.
I—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

Re-epithelialization

Keratinocytes

Type III collagen now type I

Fibroblast

Apoptosis vs. Synthesis balance

Excessive fibrosis lead to scar

Inflammation

Neutrophils

Macrophages

Fibroblasts

Lymphocytes

Relative Number of Cells

Days Postwounding

0 2 4 6 8 10 12

0 14 16

Surgeries of North America

Volume 77 • Number 3 • June 1997

509
Wound Healing

Maturation
Proliferation
Inflammation

Days Postwounding

Relative Amounts of Matrix Synthesis

Fibronectin
Collagen III
Collagen I
Wound breaking strength

VOLUME 77 • NUMBER 3 • JUNE 1997
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>
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%\textsuperscript{23}
• FGF
  – Venous ulcers, diabetic wounds\textsuperscript{24}
    • 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

Outcomes measures
- Closure
- Hearing Level
- Sx Sequela

N = 56
63 TMP

53 bFGF

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

10 gelfilm
Regenerative Treatment for Tympanic Membrane Perforation
Regenerative Treatment for Tympanic Membrane Perforation

Results
Regenerative Treatment for Tympanic Membrane Perforation

Results

[Graph showing dB levels before and 3 months after treatment across different kHz frequencies]
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**


<table>
<thead>
<tr>
<th>Mild or moderate oral mucositis = Grades 1 and 2 (WHO)</th>
<th>Severe oral mucositis = Grades 3 and 4 (WHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1 Erythema Unpleasant sensation (pain)</td>
<td>Grade 3 Ulcers Significant pain Only a liquid diet is possible</td>
</tr>
<tr>
<td>Grade 2 Erythema Ulcers Pain Can eat solids</td>
<td>Grade 4 Ulcers Intolerable pain Feeding by mouth impossible, enteral or parenteral feeding obligatory Cannot talk</td>
</tr>
</tbody>
</table>

---

The NEW ENGLAND JOURNAL of MEDICINE

Jefferson University Hospital

BEST HOSPITALS
USNews
HONOR ROLL
2013-14
Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers


N = 212

3x IV Palifermin

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

3x IV Placebo

3x additional doses after BMT

Hodkin’s Disease
Non-Hodgkin’s Lymphoma
Leukemia
Multiple Myeloma
### Table 2. Effect of Palifermin on Oral Mucositis of WHO Grade 3 or 4 and Patient-Reported Outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Palifermin Group (N=106)</th>
<th>Placebo Group (N=106)</th>
<th>P Value*</th>
</tr>
</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>Duration — days</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>
</tr>
<tr>
<td>Swallowing-limitation score</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median</td>
<td>22.5</td>
<td>38.3</td>
<td></td>
</tr>
<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></td>
</tr>
<tr>
<td>Median</td>
<td>736.6</td>
<td>712.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Range</td>
<td>176–1033</td>
<td>176–1014</td>
<td></td>
</tr>
<tr>
<td>Functional well-being domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>546.1</td>
<td>542.5</td>
<td>0.035</td>
</tr>
<tr>
<td>Range</td>
<td>93–985</td>
<td>93–1043</td>
<td></td>
</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
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)
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

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

[Diagram of surgical procedure]
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

N = 68
2005-2008

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

PRP-Scaffold Rhinoplasty
Mean follow up 15 months

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”
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>Epicel</td>
</tr>
<tr>
<td>Acellular free-dried cadaveric skin allograft</td>
<td>Dermal</td>
<td>No</td>
<td>AlloDerm</td>
</tr>
<tr>
<td>Bovine collagen/glycosaminoglycan/Silastic</td>
<td>Dermal</td>
<td>No</td>
<td>Integra</td>
</tr>
<tr>
<td>Neonatal fibroblasts/polyglactin mesh allograft</td>
<td>Dermal</td>
<td>Yes</td>
<td>Dermagraft</td>
</tr>
<tr>
<td>Neonatal fibroblasts/keratinocytes collagen allograft</td>
<td>Composite</td>
<td>Yes</td>
<td>Apligraf</td>
</tr>
</tbody>
</table>
Bioengineered skin: Apligraf

Development of a bilayered living skin construct for clinical applications.
Wilkins LM¹, Watson SR, Prosky SJ, Meunier SF, Parenteau NL.

- 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
Bioengineered skin: Apligraf
Hyperbaric Oxygen
Hyperbaric Oxygen

1 atm
55mm Hg

3 atm
500mm Hg

1 atm
100mm Hg

3 atm
2000mm Hg

21% oxygen diffuses into the surrounding tissues from the red blood cells.

Hyperbaric oxygen: its mechanisms and efficacy.
Thom 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 PRE-CONDITIONING 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. Lefaivre, 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”
Dr. Harvey Cushing

From A Surgeon's Journal

HARVEY CUSHING
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
- Defect
- Pedicle
- 3 cm
- 8 cm
Pre Op
Post Op
Old vs. Young

B.
Histopathology

Young

Old
A.

**Flap segment**

- P
- M
- D

**IB:**

- VEGF

**Days after wounding**

- 0
- 2
- 5
- 9

**Old**

**Young**
Increasing Akt activation
Apoptosis

Flap segment

<table>
<thead>
<tr>
<th>IB:</th>
</tr>
</thead>
<tbody>
<tr>
<td>←VEGF-A$_{189}$</td>
</tr>
<tr>
<td>←VEGF-A$_{165}$</td>
</tr>
<tr>
<td>←VEGF-A$_{121}$</td>
</tr>
<tr>
<td>←VEGF-A mature</td>
</tr>
<tr>
<td>p-VEGFR-2 (Y1059)</td>
</tr>
<tr>
<td>p-Akt (S473)</td>
</tr>
<tr>
<td>p-eNOS (S1177)</td>
</tr>
<tr>
<td>Caspase-3 (CL)</td>
</tr>
<tr>
<td>Bcl-2</td>
</tr>
<tr>
<td>Bax</td>
</tr>
<tr>
<td>←PARP (FL)</td>
</tr>
<tr>
<td>←PARP (CL)</td>
</tr>
<tr>
<td>GAPDH</td>
</tr>
</tbody>
</table>

Animal groups

Young | Old
Initial Conclusions

• Young vs. old
  – Increased VEGF
  – Increased Atk
  – Decreased apoptosis

What this really means….

• 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)

YR

YE

0 | 2 days | 5 days | 9 days

Time post-surgery
Exercise Old Rats

Flap area

IB:

- p-Akt (S473)
- Akt (pan)

OR

OE

Time post-surgery

0  2 days  5 days  9 days
VEGF

Flap area and day of tissue collection after surgery

- Old Resting (n=6)
- Old Exercising (n=6)
- Young Resting (n=6)
- Young Exercising (n=5)
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