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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
The Great War
Carrel Apparatus
Carrel-Dakins solution

APPARATUS FOR APPLYING CARREL-DAKINS 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.
J—Flask for use with syringe.
Use of Carrel Apparatus
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

Deep wound healing

- Scab
- Resurfaced epithelium
- Collagen fibers
- Scar tissue
- Fibroblast
- Restored blood vessel

Maturation phase
Wound Healing

Hemostasis:

Platelet activation

Cellular influx

Inflammation

Inflammation:

Neutrophils

Macrophages

Fibroblasts

Lymphocytes

Relative Number of Cells

Days Postwounding

0 2 4 6 8 10 12 14 16

Maturation

Proliferation

Re-epithelialization

Keratinocytes

Fibroblast

Type III collagen now type I

Apoptosis vs. Synthesis balance

Excessive fibrosis lead to scar

Excessive fibrosis lead to scar

Type III collagen now type I

Apoptosis vs. Synthesis balance

Excessive fibrosis lead to scar
Wound Healing

- Inflammation
- Proliferation
- Maturation

Graph showing the relative amounts of matrix synthesis over days postwounding:
- Collagen I: increasing from day 0 to 16
- Collagen III: increasing from day 0 to 16
- Fibronectin: decreasing from day 0 to 4

Wound breaking strength also increases over time.
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

Platelet plug

Epidermis

Neutrophil

Blood vessel

VEGF

FGF-2

Dermis

Fat

FGF

VEGF

PDGF BB

IGF

KGF

PDGF

TGF-β1

TGF-α

Macrophage

Fibroblast

TGF-β1

TGF-β2

TGF-β3

PDGF AB

FGF-2

TGF-β1

Fibroblast
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

- N = 56
- 63 TMP

Outcomes measures:
- Closure
- Hearing Level
- Sx Sequela

53 bFGF

10 gelfilm

TMP s/p OM inflammation
Old traumatic TMP
TMP s/p tube
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
Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers


N = 212

3x IV Placebo

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

3x IV Palifermin

N = 212

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

3x additional doses after BMT
### 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>
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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>
</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>Functional Assessment of Cancer Therapy general score</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>
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<tr>
<td>Range</td>
<td>176–1033</td>
<td>176–1014</td>
<td></td>
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<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>0.036</td>
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<tr>
<td>Range</td>
<td>93–985</td>
<td>93–1043</td>
<td></td>
</tr>
</tbody>
</table>

Severe Mucositis

- **63% vs. 98%**
- **3 days vs. 9 days**
- **29 vs. 46**
- **22.5 vs. 38.3**
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)

CaCl₂
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>
<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>

*ELISA*
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
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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

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

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”
Other applications…

**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, décolleté, 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 (cultured epidermal autografts)</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>

---

*Image from Jefferson University Hospital*
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

55 mm Hg

100 mm Hg

3 atm

500 mm Hg

2000 mm Hg

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


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

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

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

11 RCT, N = 669

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
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
- a
Pre Op
Post Op
Old vs. Young

B.

Percent of necrosis (%)
Histopathology

Young

Old
VEGF

A. Flap segment

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>M</th>
<th>D</th>
<th>P</th>
<th>M</th>
<th>D</th>
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<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Young</td>
<td></td>
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</tr>
</tbody>
</table>

Days after wounding

IB: -VEGF
Increasing Akt activation
### Apoptosis

#### Flap segment

<table>
<thead>
<tr>
<th>Flap segment</th>
<th>P</th>
<th>M</th>
<th>D</th>
<th>P</th>
<th>M</th>
<th>D</th>
</tr>
</thead>
</table>

#### IB:

- VEGF-A$_{189}$
- VEGF-A$_{165}$
- VEGF-A$_{121}$
- VEGF-A mature
- p-VEGFR-2 (Y1059)
- p-Akt (S473)
- p-eNOS (S1177)
- Caspase-3 (CL)
- Bcl-2
- Bax
- PARP (FL)
- PARP (CL)
- GAPDH

#### 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

- P M D P M D P M D

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

0 | 2 days | 5 days | 9 days

Time post-surgery
Exercise Old Rats

Flap area

IB:

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

OR
- p-Akt (S473)
- Act (pan)

OE

<table>
<thead>
<tr>
<th>Time post-surgery</th>
<th>P</th>
<th>M</th>
<th>D</th>
<th>P</th>
<th>M</th>
<th>D</th>
<th>P</th>
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<td>2 days</td>
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<td>5 days</td>
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<tr>
<td>9 days</td>
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<td></td>
</tr>
</tbody>
</table>
VEGF

Flap area and day of tissue collection after surgery
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