3-26-2014

Physiology and Advancements in Wound Healing

Adam Baker, MD
Thomas Jefferson University

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

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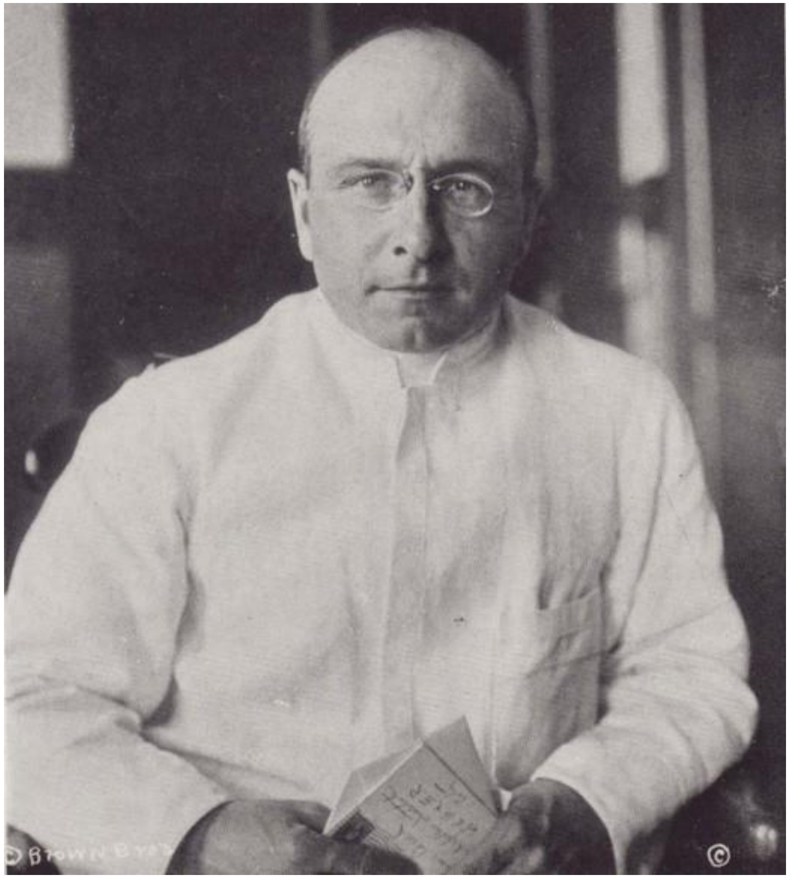
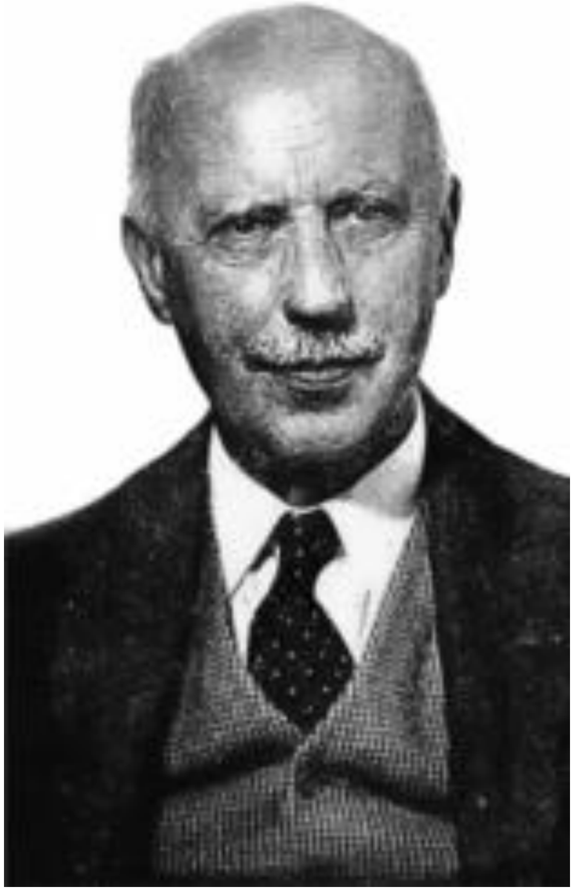
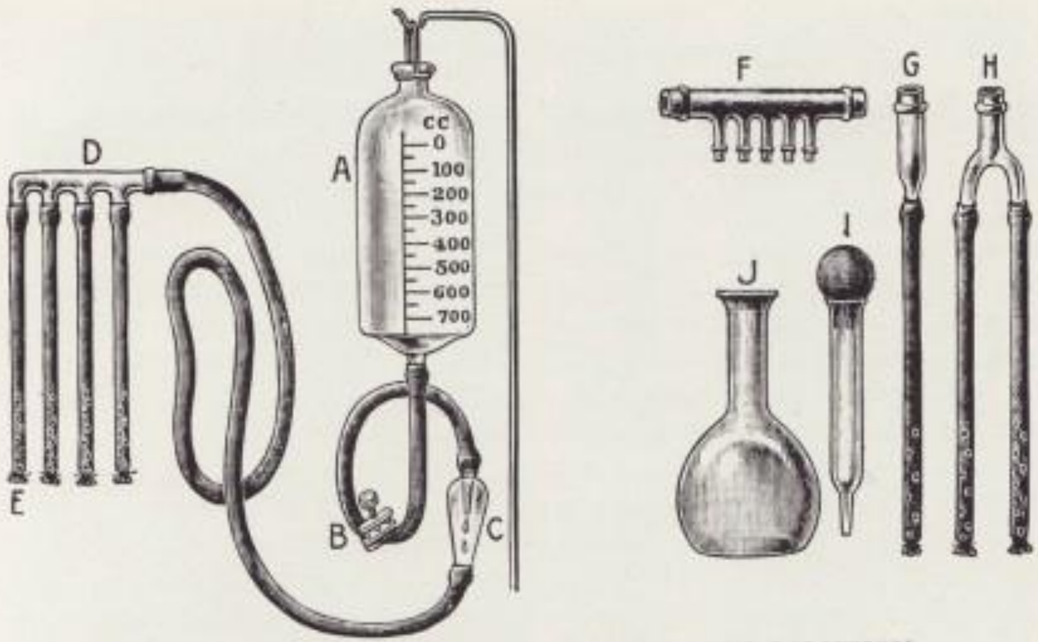


Alexis Carrel, MD



Henry Drysdale Dakin, PhD

Carrel Apparatus
Carrel-Dakins solution

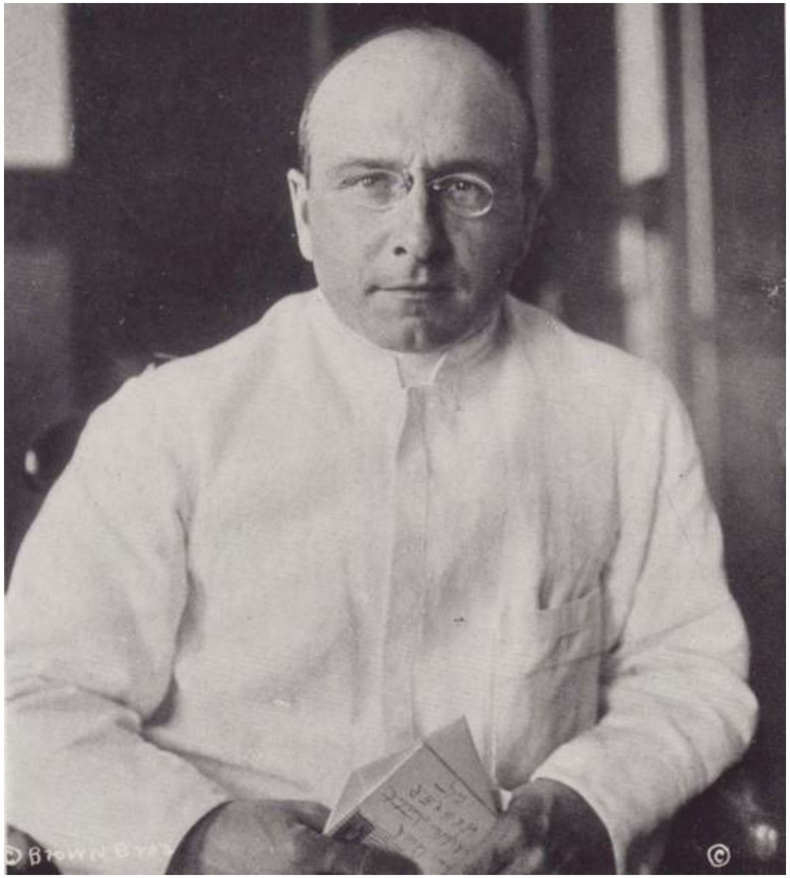


APPARATUS FOR APPLYING CARREL-DAKIN SOLUTION

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



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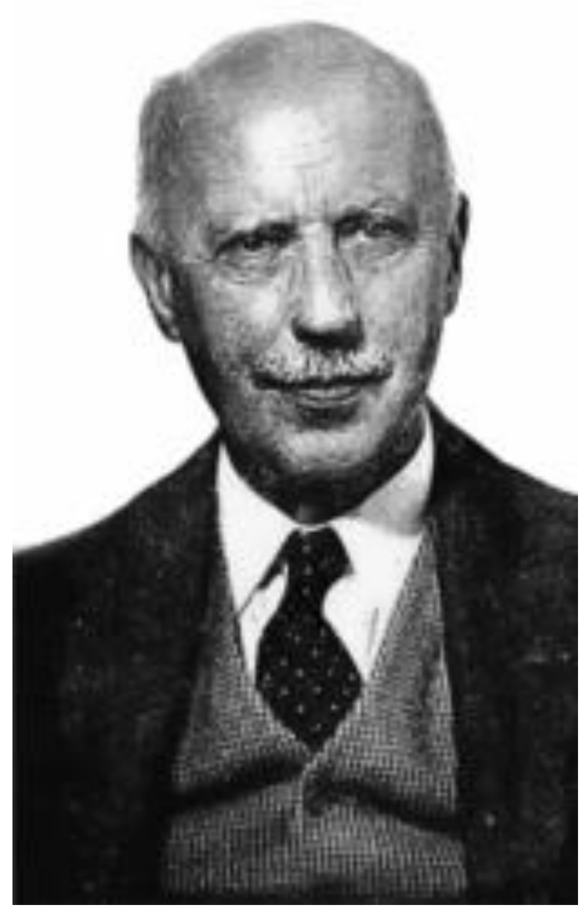
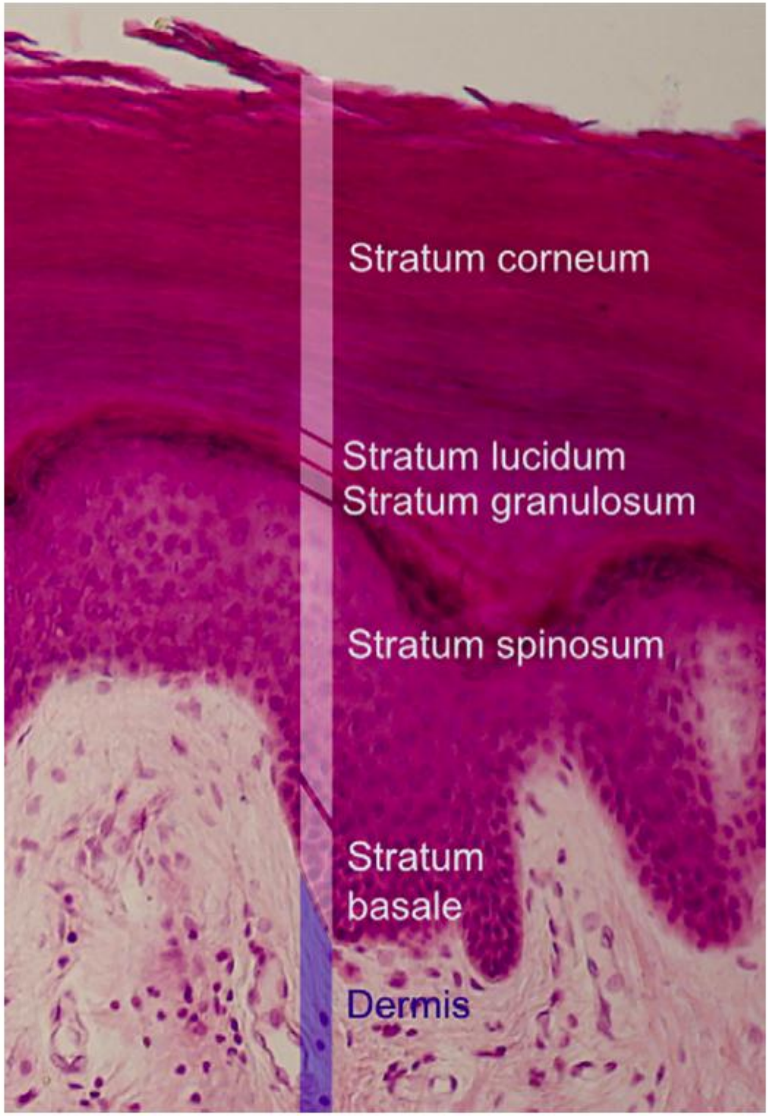


Henry Drysdale Dakin, PhD

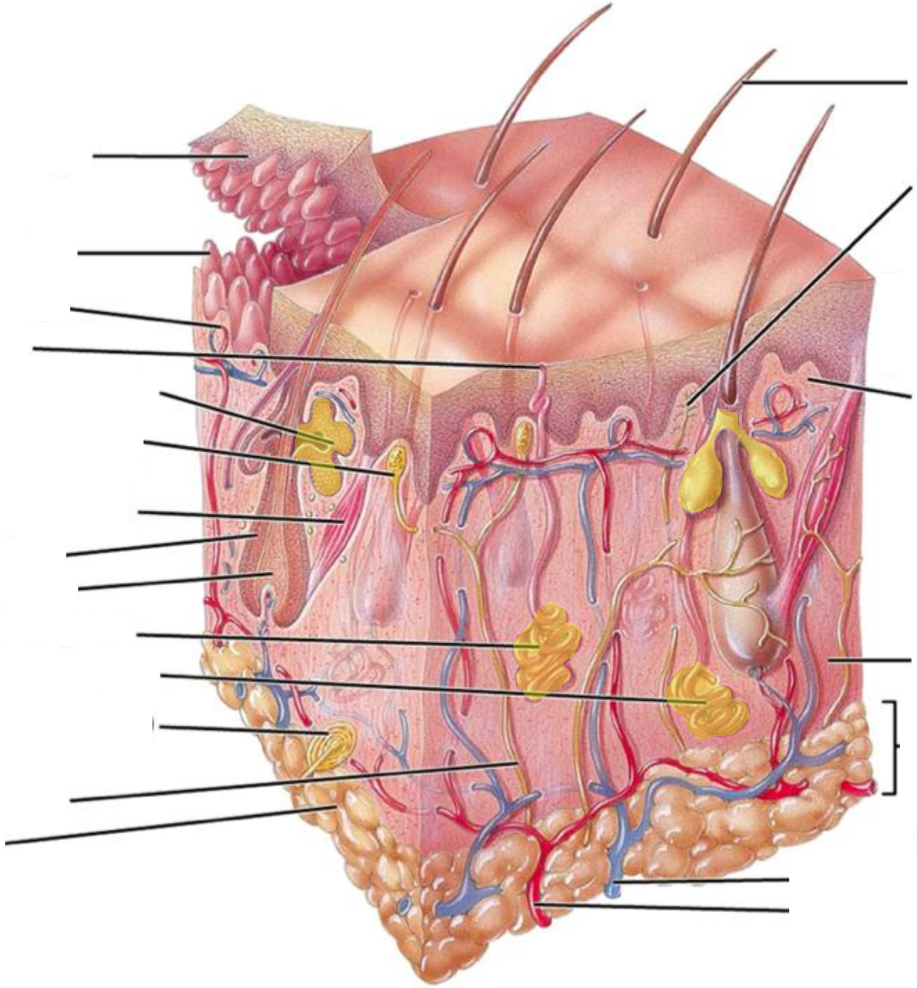
Skin Anatomy

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



Clin Plastic Surg 39 (2012) 85–97
doi:10.1016/j.cps.2011.09.005

Pilosebaceous Unit



Sectional view of skin and subcutaneous layer

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

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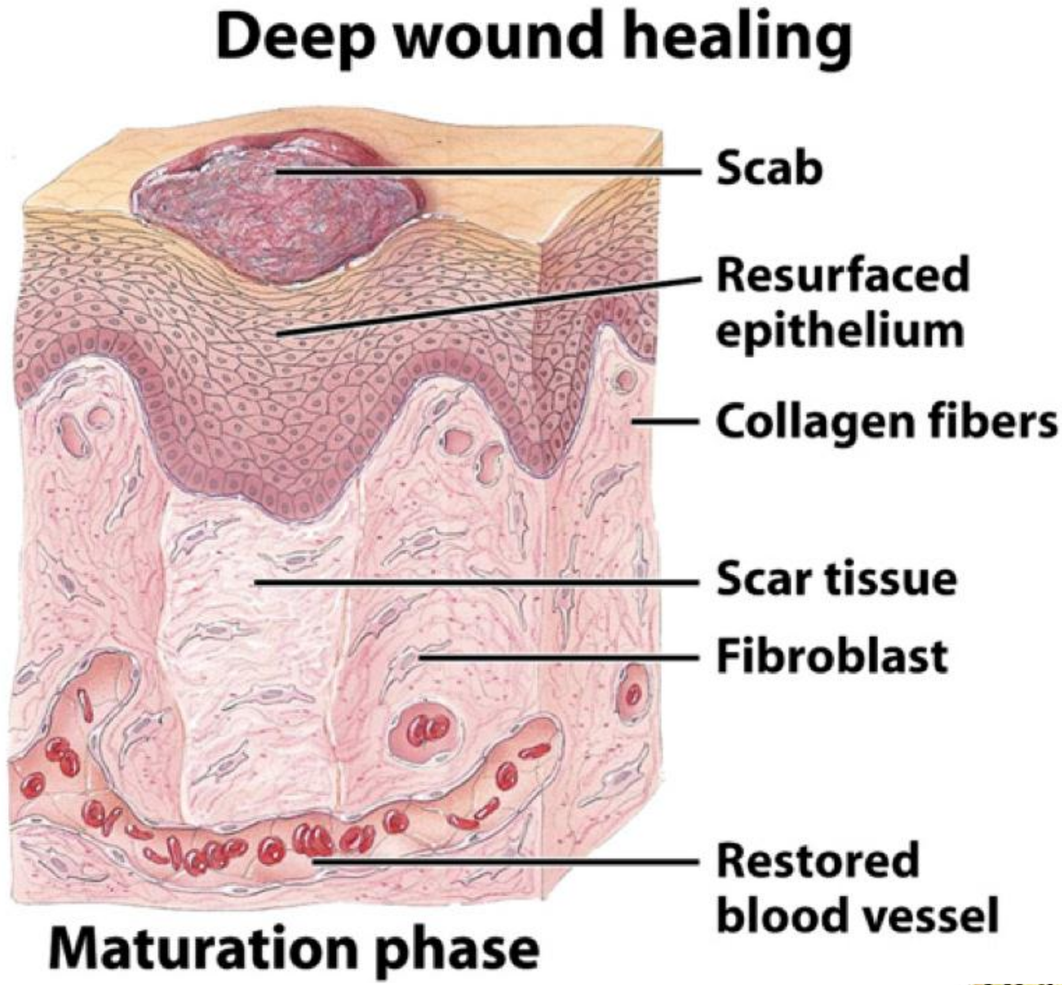
doi:10.1016/j.cps.2011.09.005

Wound Healing



- Phases
  - Hemostasis-Inflammation
  - Proliferation
  - Maturation-Remodeling

Clin Plastic Surg 39 (2012) 85–97
doi:10.1016/j.cps.2011.09.005

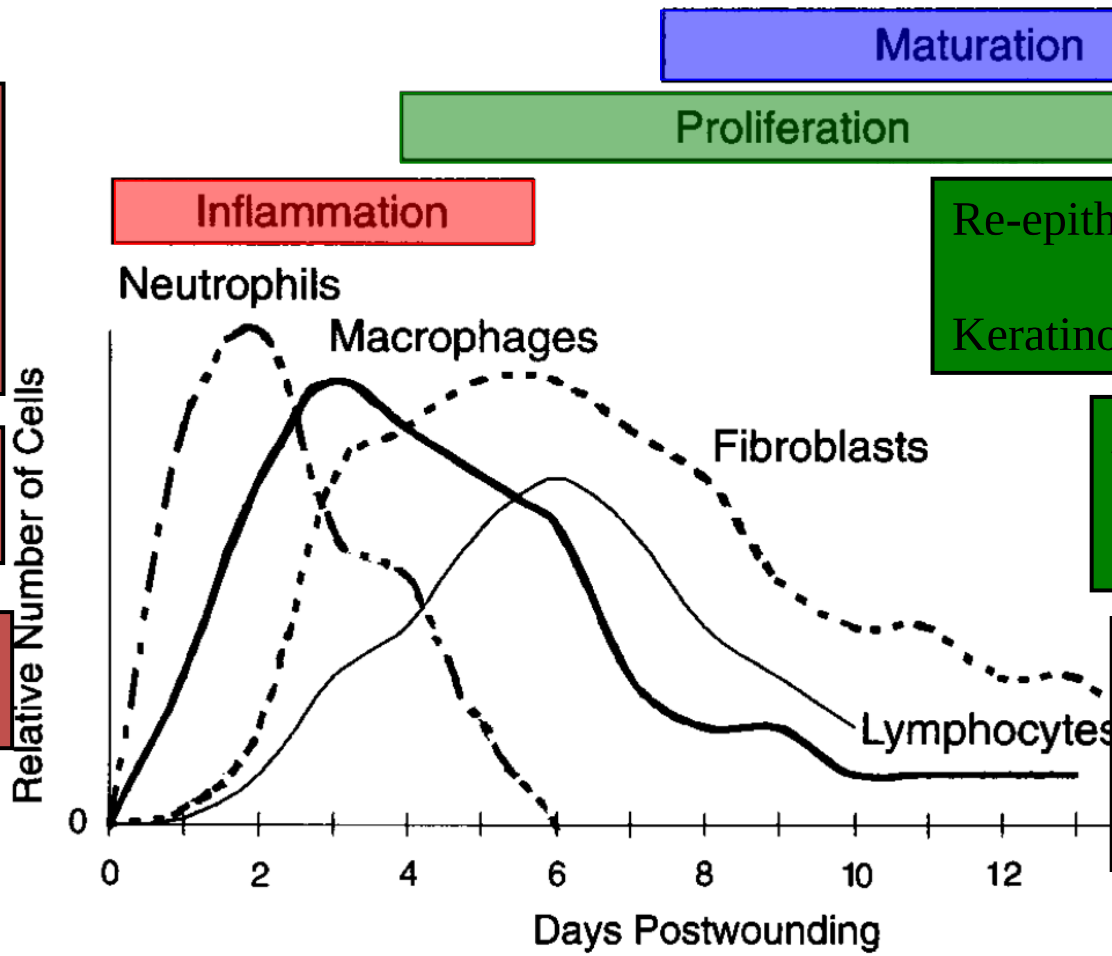
Wound Healing

Hemostasis:

platelet activation

Cellular influx

Inflammation



Re-epithe

Keratinoc

Type III collagen now type I

Fibroblast

Apoptosis vs. Synthesis balance

Excessive fibrosis lead to scar

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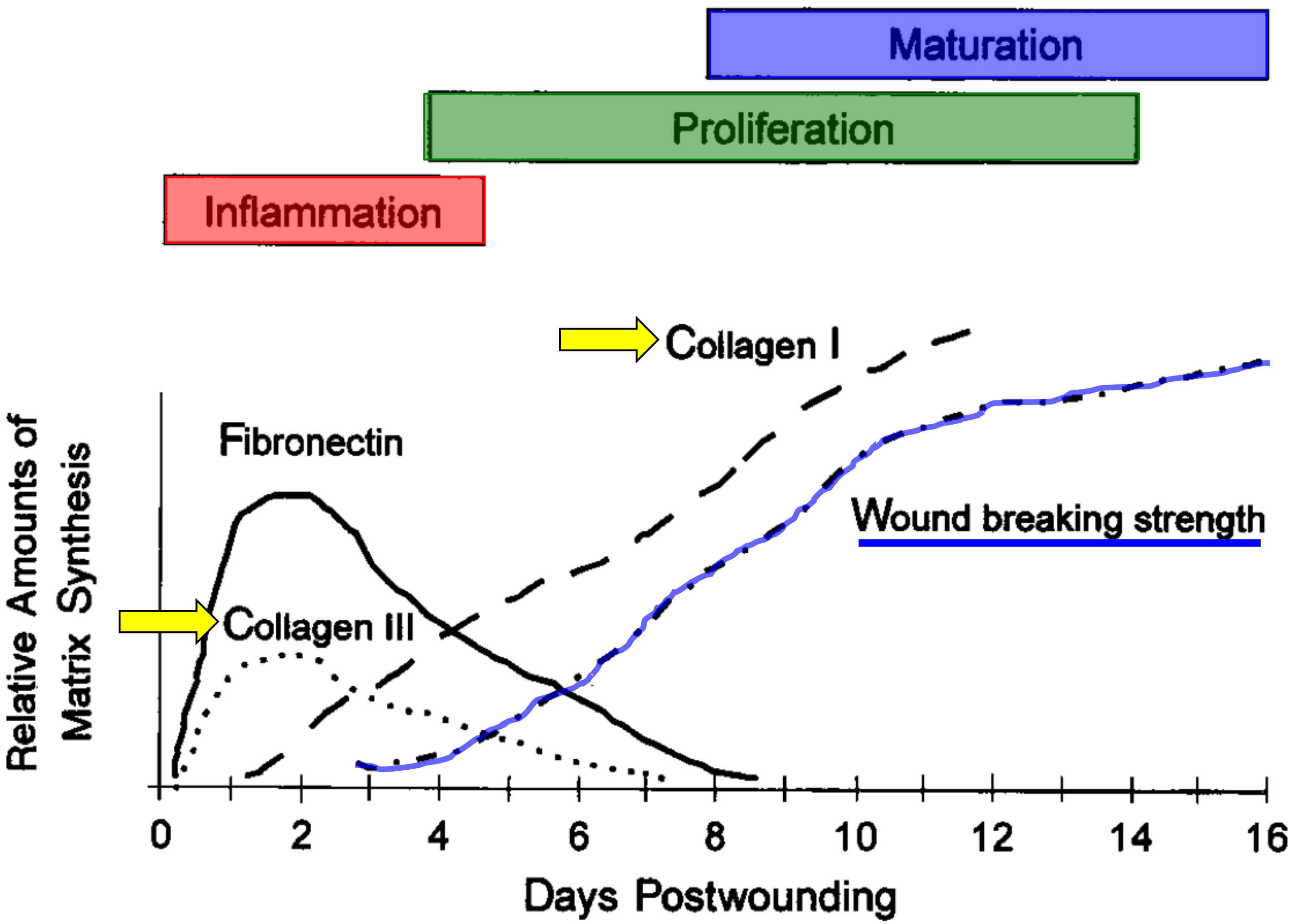
VOLUME 77 • NUMBER 3 • JUNE 1997 509

Wound Healing



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Macrophage: The QB

Macrophage

SURGICAL CLINICS OF NORTH AMERICA

VOLUME 77 • NUMBER 3 • JUNE 1997 509

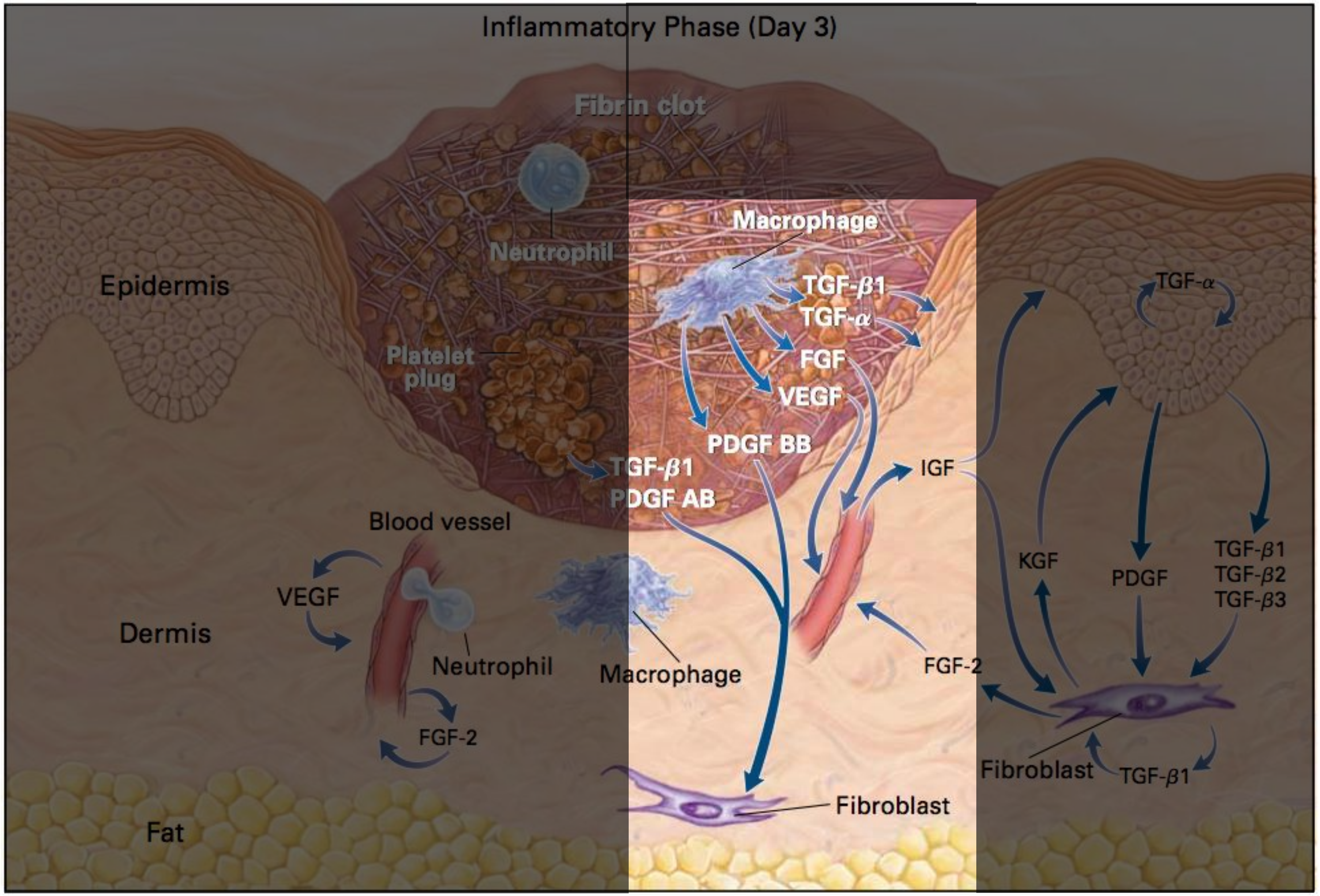
Wound Healing: Growth Factors

Growth Factor	Biologic effect
Platelet-derived growth factor (PDGF)	Proliferation, chemotaxis, matrix synthesis
Transforming growth factor- (TGF)	Inflammation, granulation
Vascular endothelial growth factor (VEGF)	Angiogenesis
Fibroblast growth factor (FGF)	Granulation, re-epith
Keratinocyte growth factor (KGF)	Re-epithelialization

Wound Rep Reg (2008) 16 585–601

585

Wound Healing: Growth Factors



Cutaneous Wound Healing
Volume 341 Number 10 · 739
Adam J. Singer, M.D., and Richard A.F. Clark, M.D.
The New England Journal of Medicine


1
2
3
4
Gunshot wound
Rib cartilage
Blood supply
Excess tissue removed
In later life
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 wounds[24]
    - Inconsistent results
  - Tympanic Membrane perforations
- KGF
  - Mucositis
- VEGF
  - Diabetic Ulcers

23. Steed DL. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity diabetic ulcers. Diabetic Ulcer Study Group. J Vasc Surg 1995;21:71–8

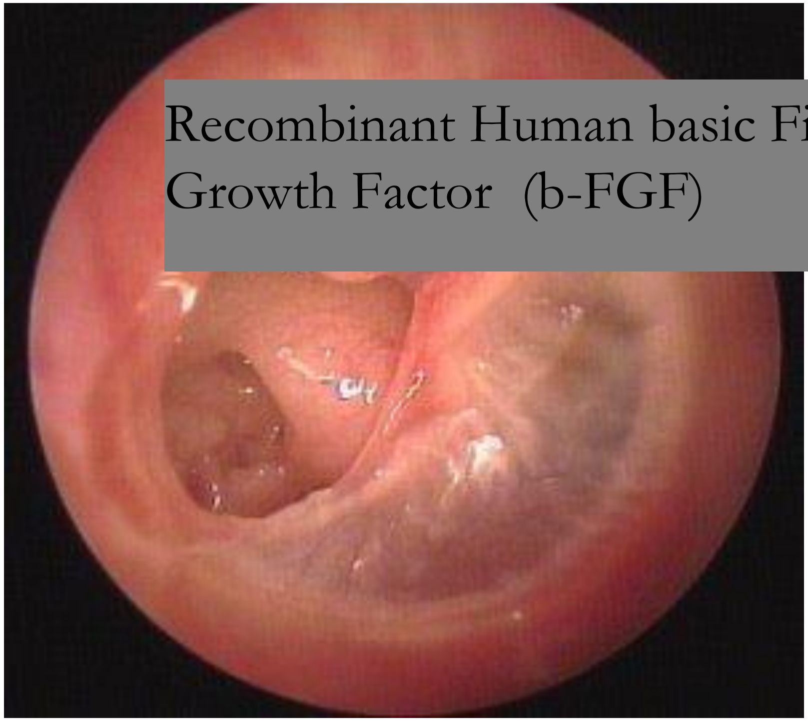
24. Robson MC, Phillips LG, Lawrence WT, et al. The safety and effect of topically applied recombinant basic fibroblast growth factor on the healing of chronic pressure sores. Ann Surg 1992;216:401–6

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)



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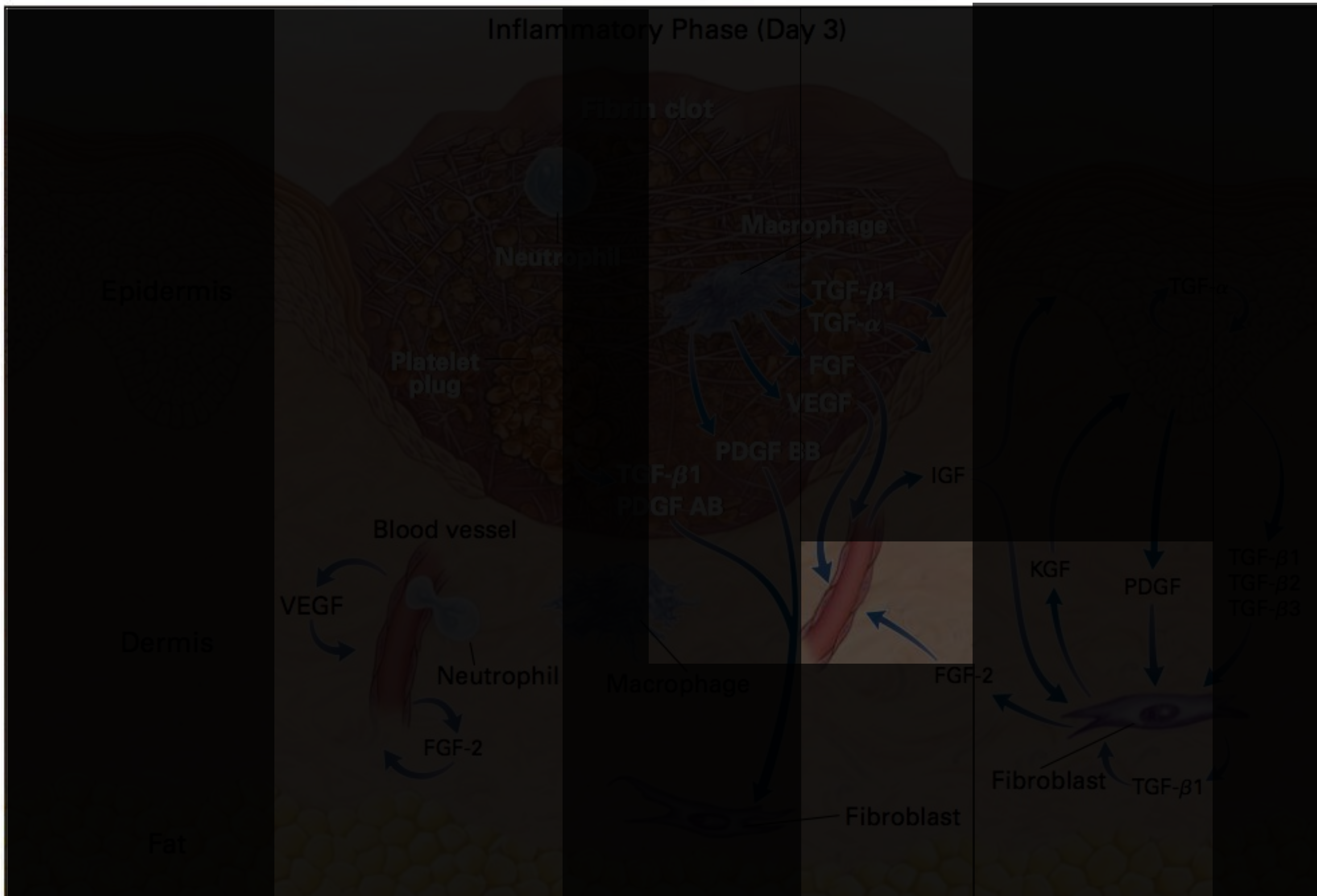

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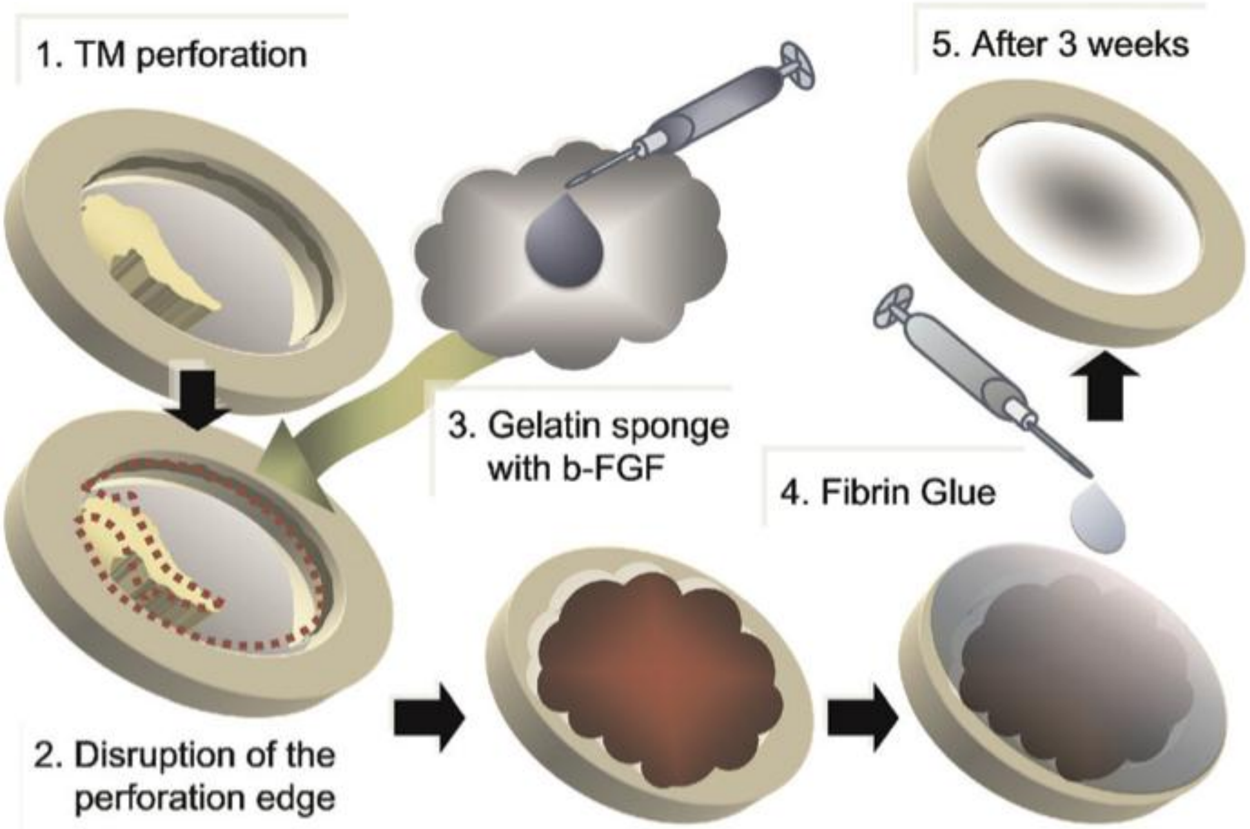
Regenerative Treatment for Tympanic Membrane Perforation



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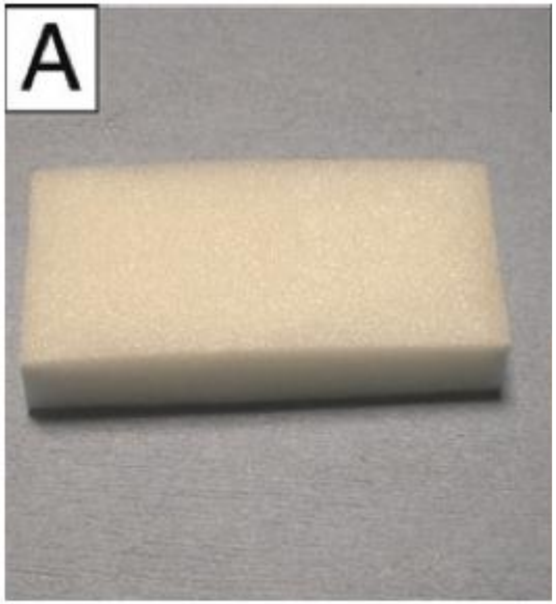

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Regenerative Treatment for Tympanic Membrane Perforation

N = 56
63 TMP

53 bFGF

10 gelfilm

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

Outcomes measures

- Closure
- Hearing Level
- Sx Sequela

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Regenerative Treatment for Tympanic Membrane Perforation

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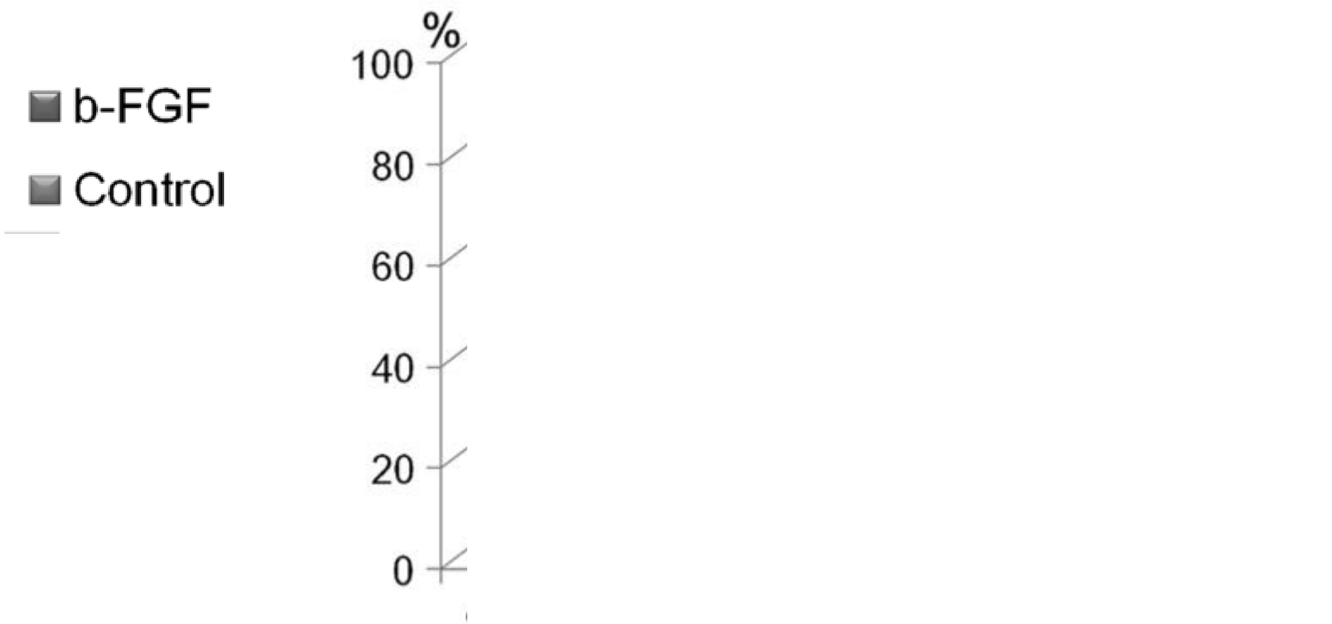
Regenerative Treatment for Tympanic Membrane Perforation

Results



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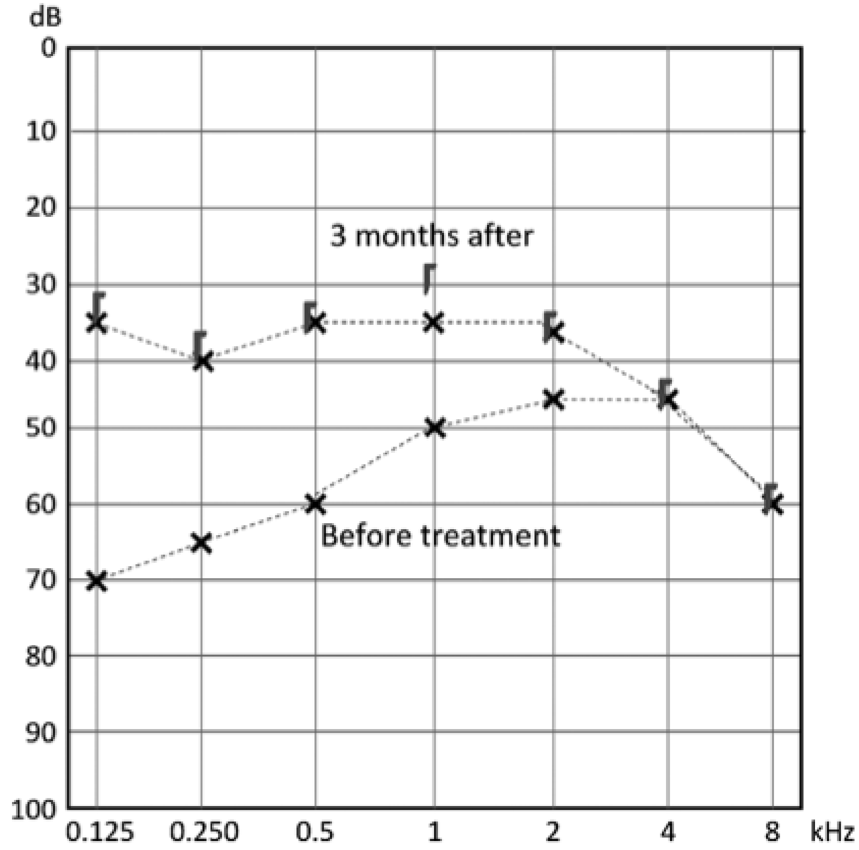
Results



The NEW ENGLAND JOURNAL of MEDICINE

Palifermin for Oral Mucositis after Intensive Therapy for Hematologic Cancers

Ricardo Spielberger, M.D., Patrick Stiff, M.D., William Bensinger, M.D.,
Teresa Gentile, M.D., Ph.D., Daniel Weisdorf, M.D., Tarun Kewalramani, M.D.,
Thomas Shea, M.D., Saul Yanovich, M.D., Keith Hansen, M.D.,
Stephen Noga, M.D., Ph.D., John McCarty, M.D., C. Frederick LeMaistre, M.D.,
Eric C. Sung, D.D.S., Bruce R. Blazar, M.D., Dieter Elhardt, Ph.D.,
Mon-Gy Chen, M.S., and Christos Emmanouilides, M.D.

NEW Kepivance™ (palifermin)

Recombinant Human Keratinocyte Growth Factor (rhKGF)

The NEW ENGLAND JOURNAL of MEDICINE

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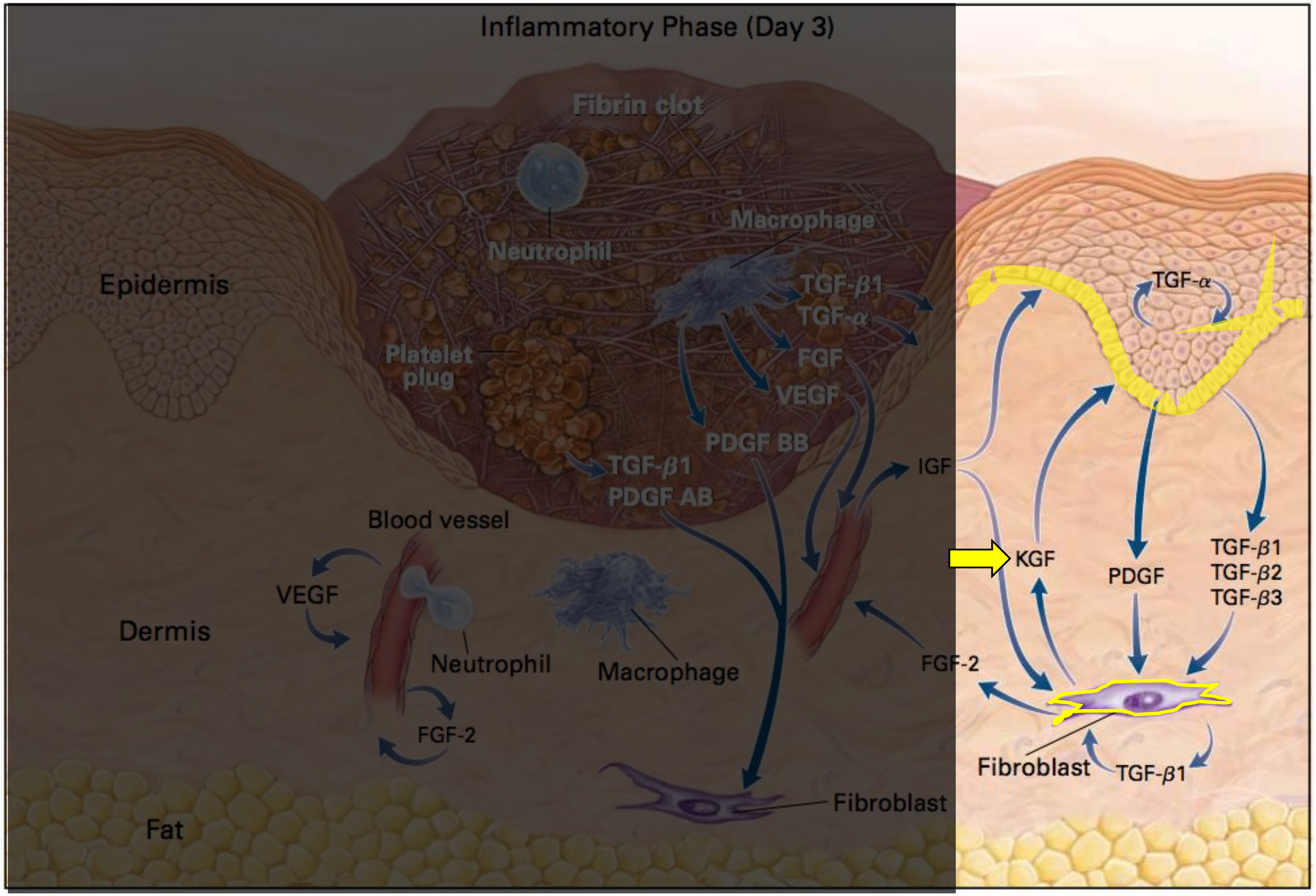

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3x IV Palifermin

N = 212

Hodkin's Disease
Non-Hodgkin's Lymphoma
Leukemia
Mutliple Myeloma

3x IV Placebo

Intensive Therapy

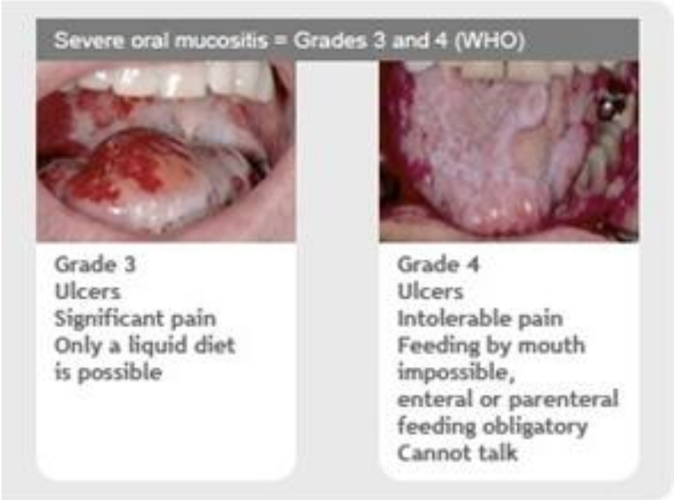
- Whole body irradiation
- Chemotherapy
- Bone Marrow Transplantation

3x additional doses after BMT

Table 2. Effect of Palifermin on Oral Mucositis of WHO Grade 3 or 4 and Patient-Reported Outcomes.

Variable	Palifermin Group (N=106)	Placebo Group (N=106)	P Value*
Oral mucositis of WHO grade 3 or 4			
Incidence — no. of patients (%)†	67 (63)	104 (98)	<0.001
Duration — days			
All patients			<0.001
Median	3.0	9.0	
Range	0–22	0–27	
Patients with oral mucositis of WHO grade 3 or 4			<0.001
Median	6.0	9.0	
Range	1–22	1–27	
Patient-reported outcomes (AUC)‡			
Score for soreness of mouth and throat			<0.001
Median	29.0	46.8	
Range	0–98	0–110	
Swallowing-limitation score			<0.001
Median	22.5	38.3	
Range	0–104	0–104	
Functional Assessment of Cancer Therapy general score			
Physical well-being domain			
Median	736.6	712.1	0.003
Range	176–1033	176–1014	
Functional well-being domain			
Median	546.1	542.5	0.036
Range	93–985	93–1043	

Severe Mucositis



63% vs. 98%

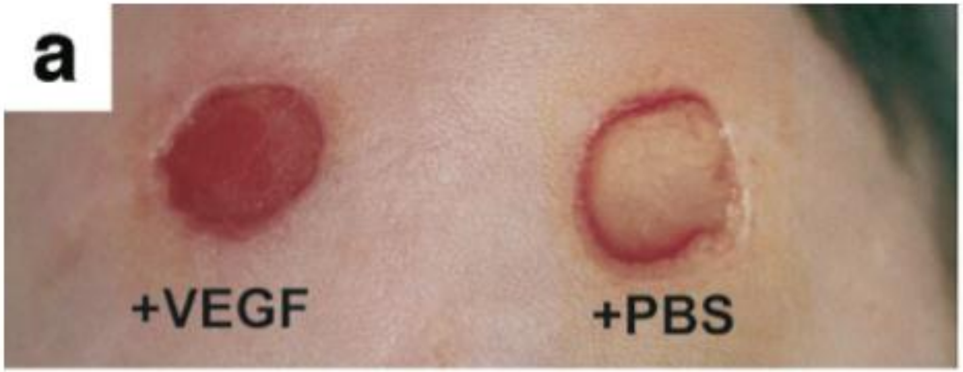
3 days vs. 9 days

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*



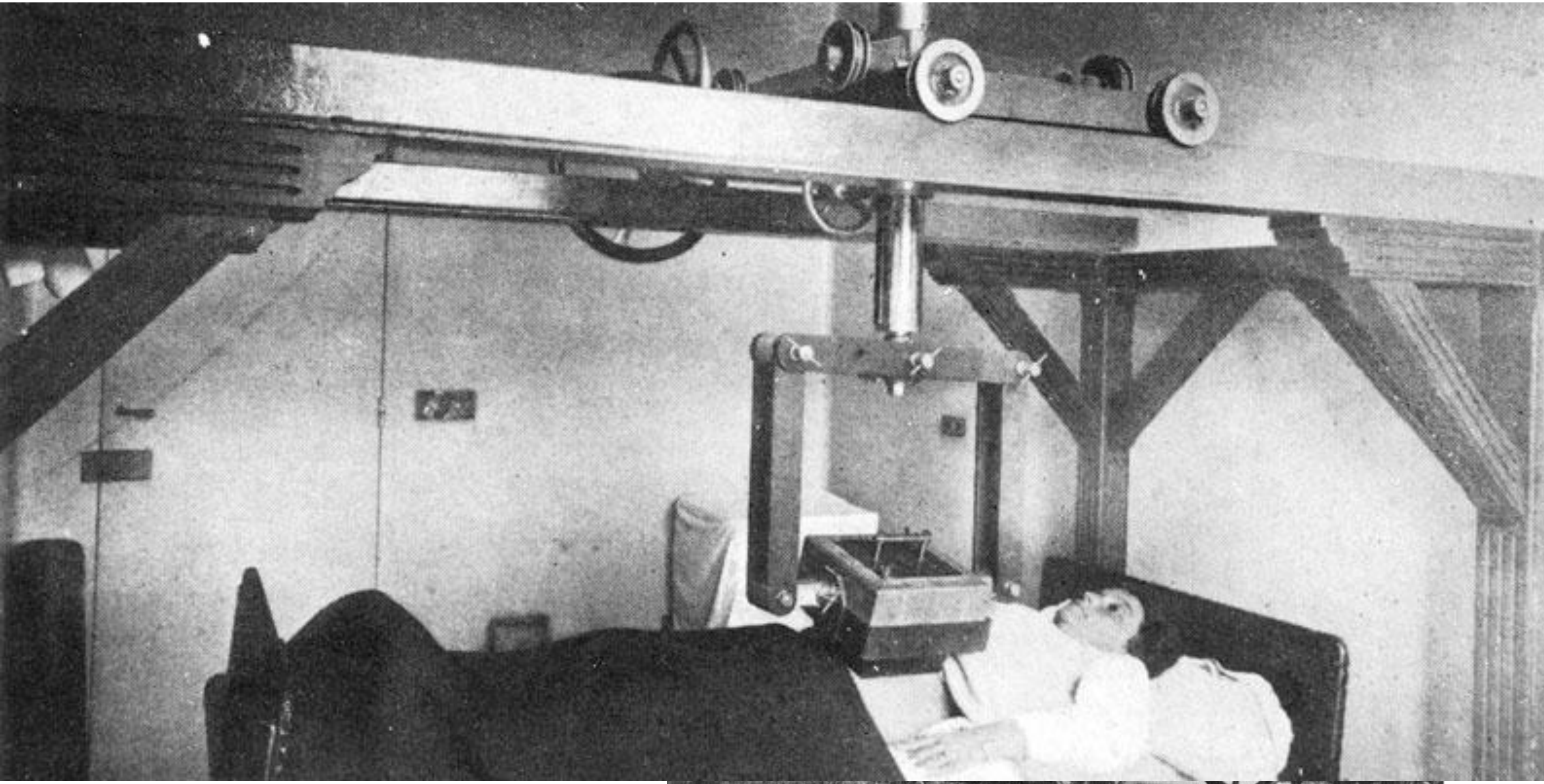
Average healing time 12 days vs. 25 days (VEGF vs control)

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

Growth Factors: Limitations

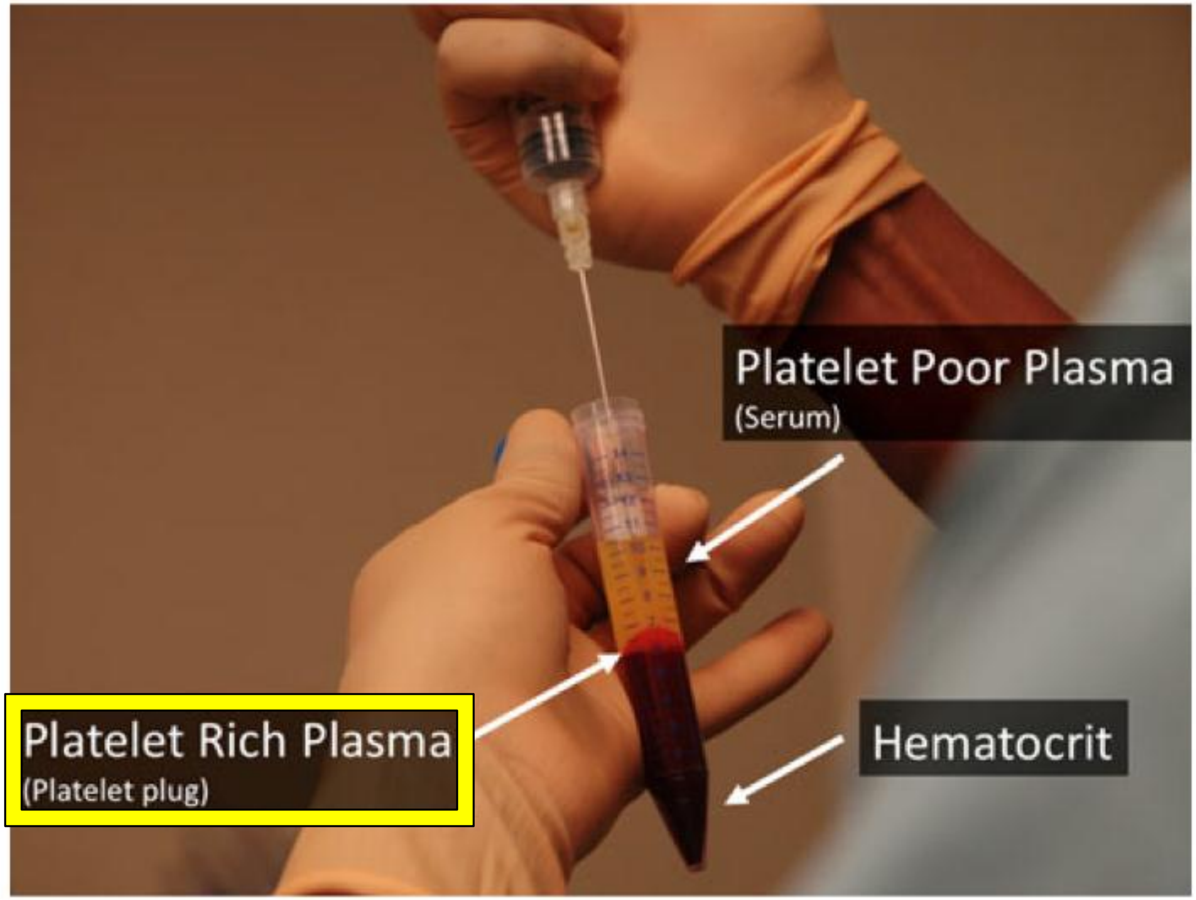
- Cost
  - Regranex $586 per 15g tube
- Delivery
  - Exception
- Risk of Malignancy
  - 2008 retrospective study
- Lack of data!

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)

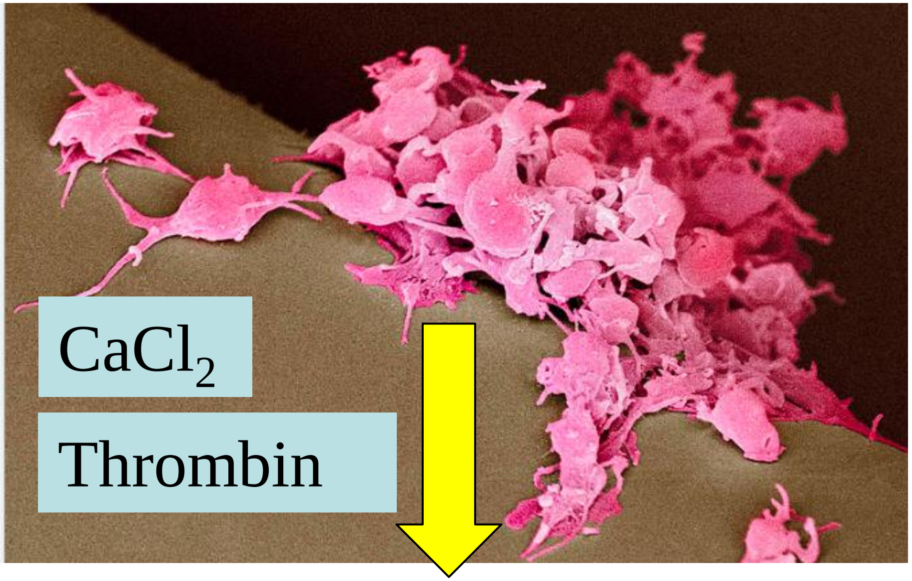
Epidermal Growth Factor
(EGF)

Transforming Growth Factor beta
(TGF-β)

Vascular Endothelial Growth Factor
(VEGF)

Fibroblast Growth Factor
(bFGF)

Epidermal Growth Factor
(EGF)



The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds

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

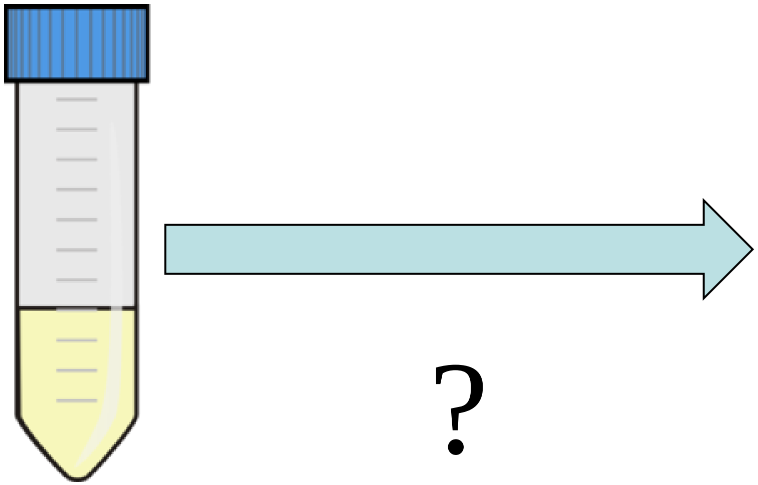


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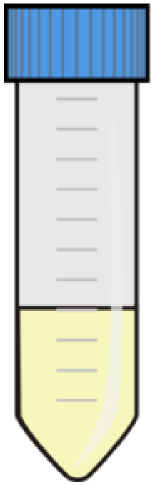
Table 3. Growth Factor Assays (ELISA)*

Growth Factors	Initial Blood Sample (60 mL)	PRP (6 mL)
PDGF-AB, ng/mL	10.2 ± 1.4	88.4 ± 28.8
PDGF-AA, ng/mL	2.7 ± 0.5	22.2 ± 4.2
PDGF-BB, ng/mL	5.8 ± 1.4	57.8 ± 36.6
TGF-β1, ng/mL	41.8 ± 9.5	231.6 ± 49.1
VEGF, pg/mL	83.1 ± 65.5	597.4 ± 431.4
bFGF, pg/mL	10.7 ± 2.9	48.4 ± 25.0
EGF, pg/mL	12.9 ± 6.2	163.3 ± 49.4



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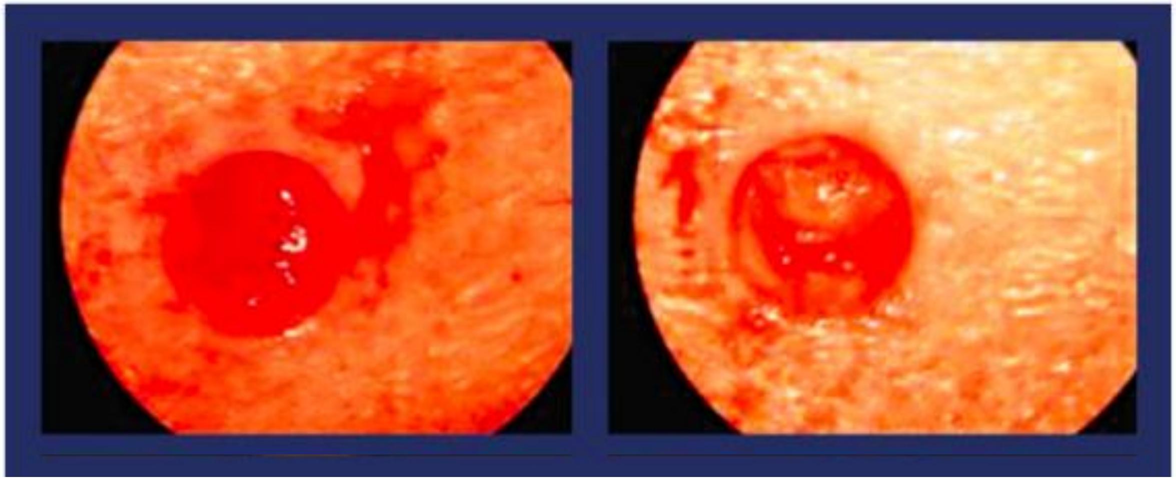
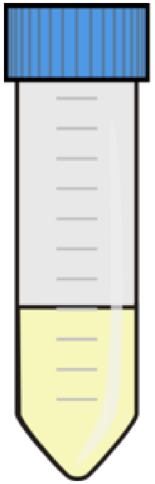
Control Site **APG-Treated Site**

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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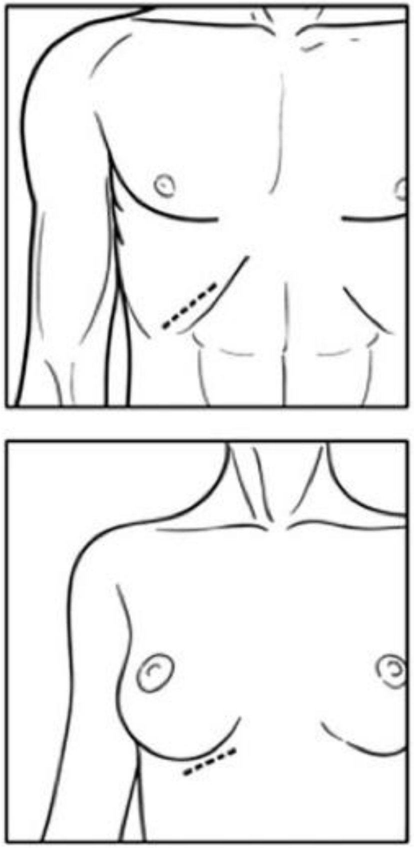

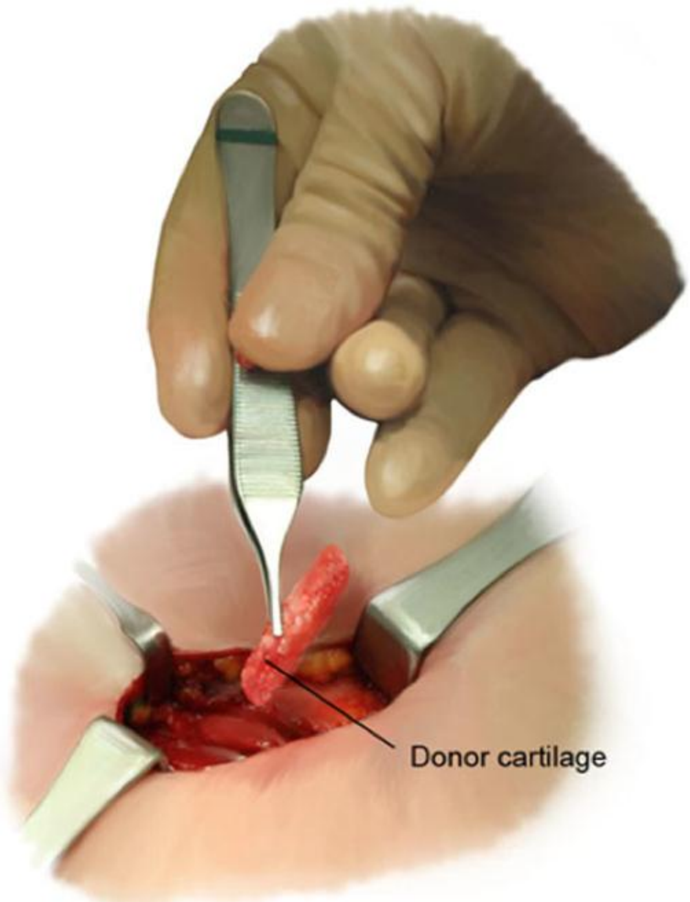

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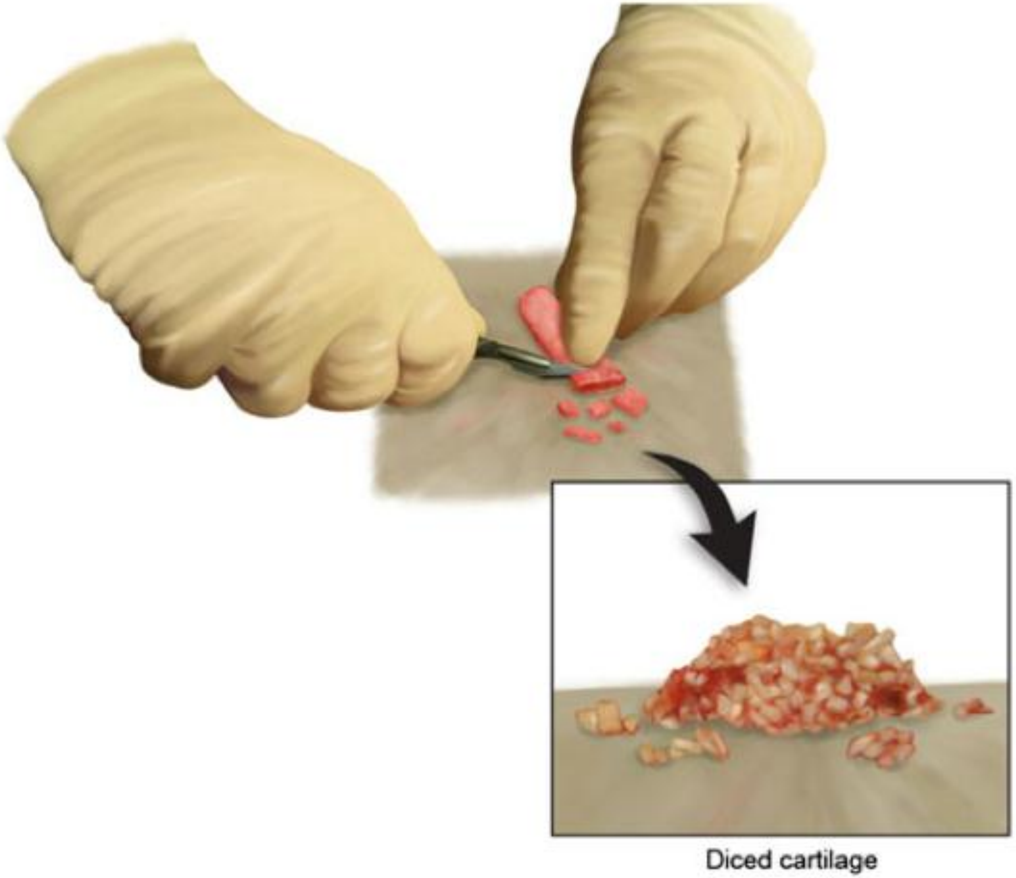

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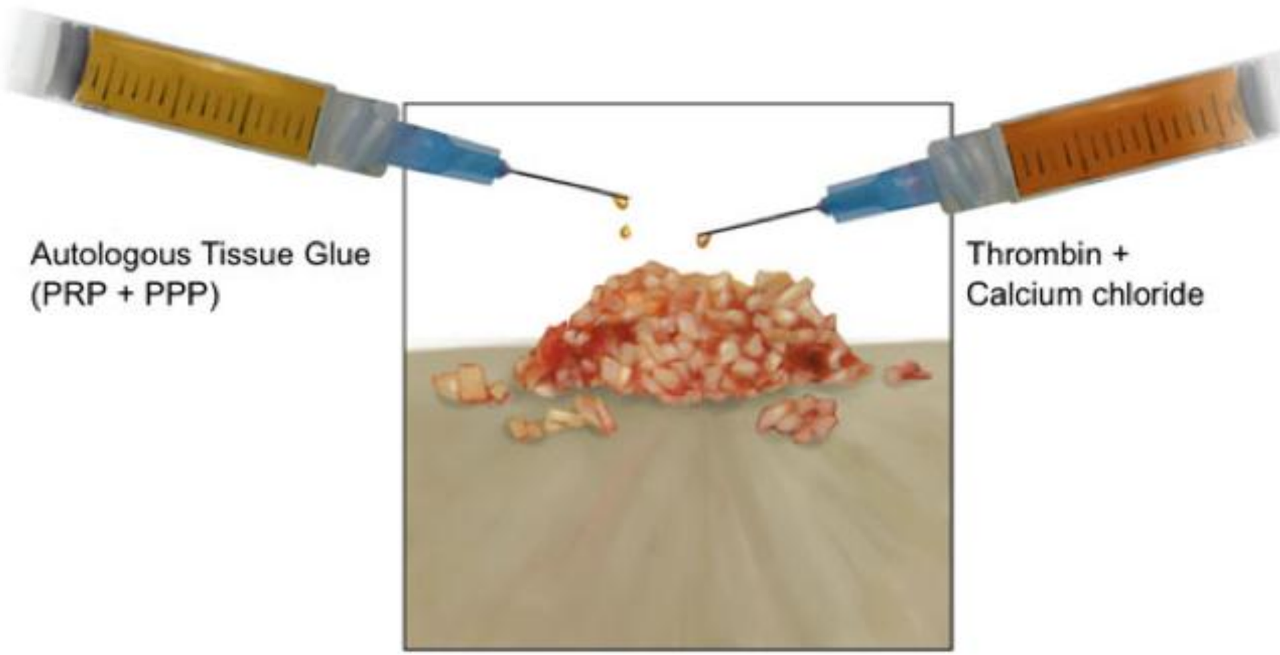

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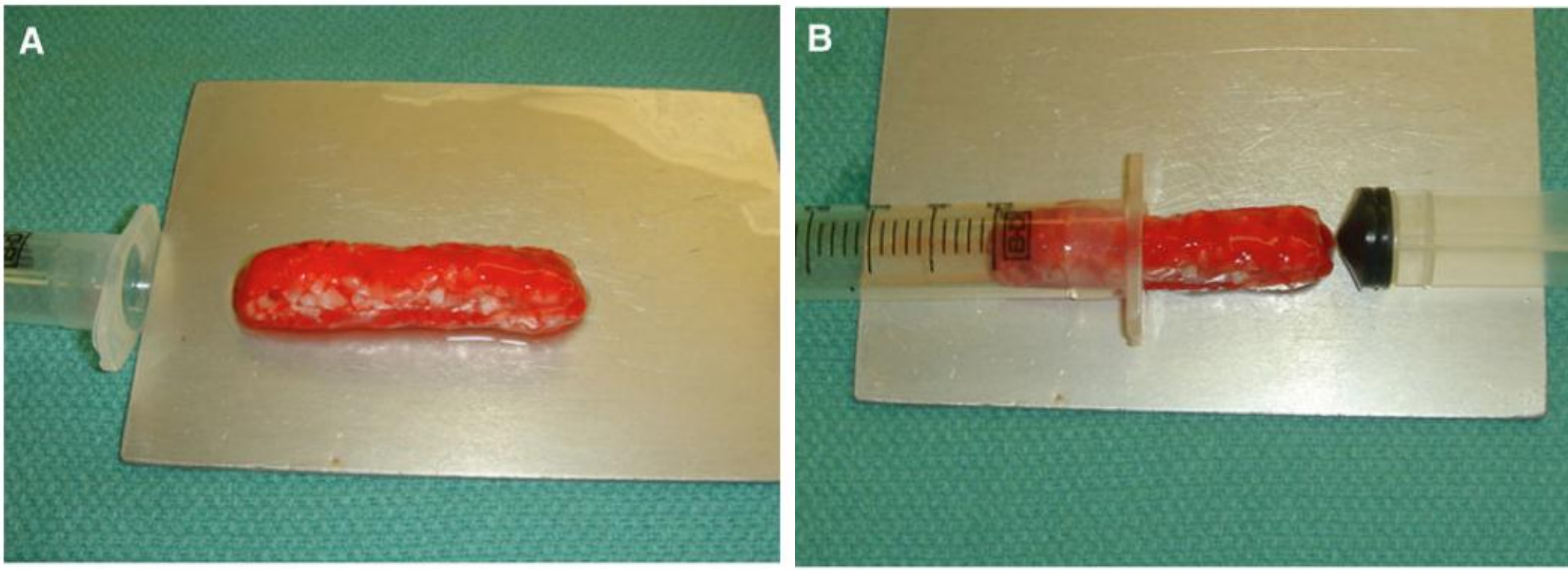

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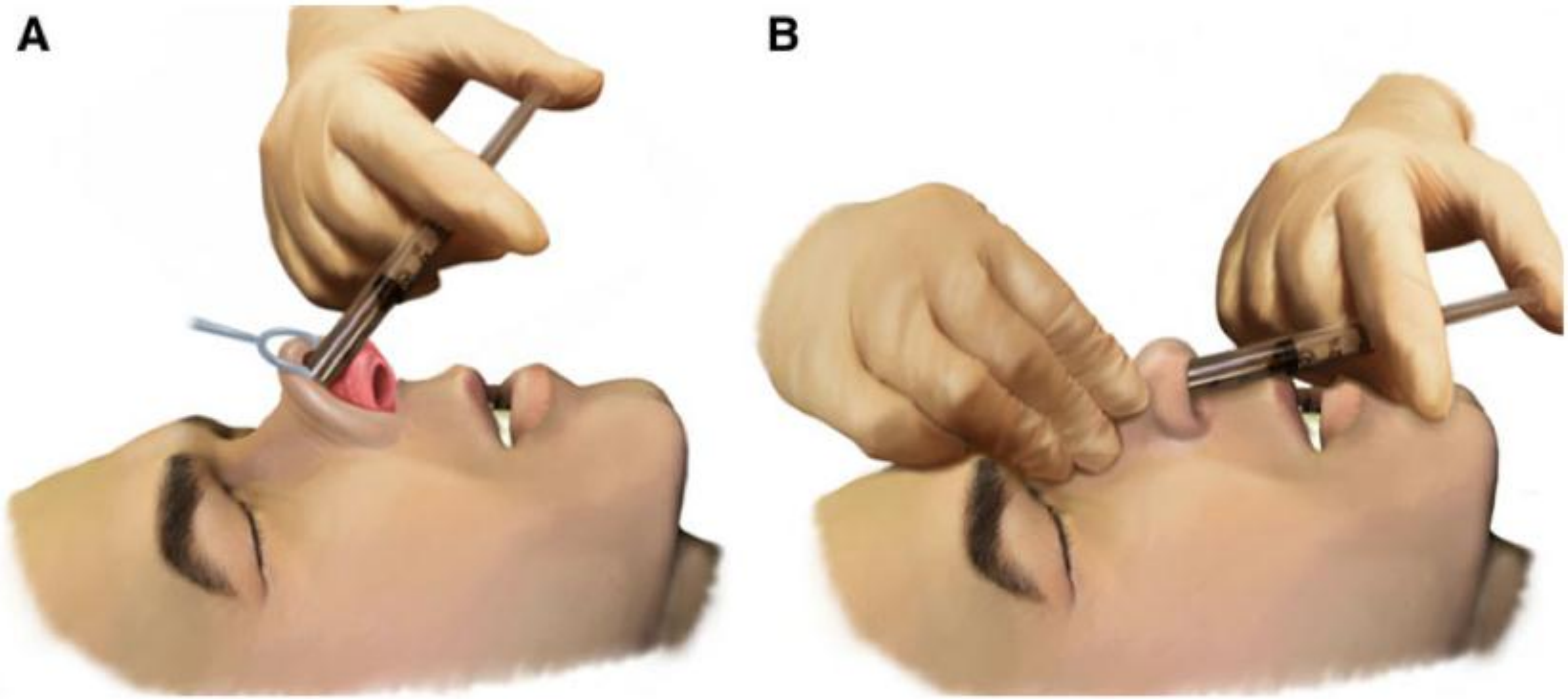


A Novel Autologous Scaffold for Diced-cartilage Grafts in Dorsal Augmentation Rhinoplasty

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

N = 68 → PRP-Scaffold Rhinoplasty

2005-2008

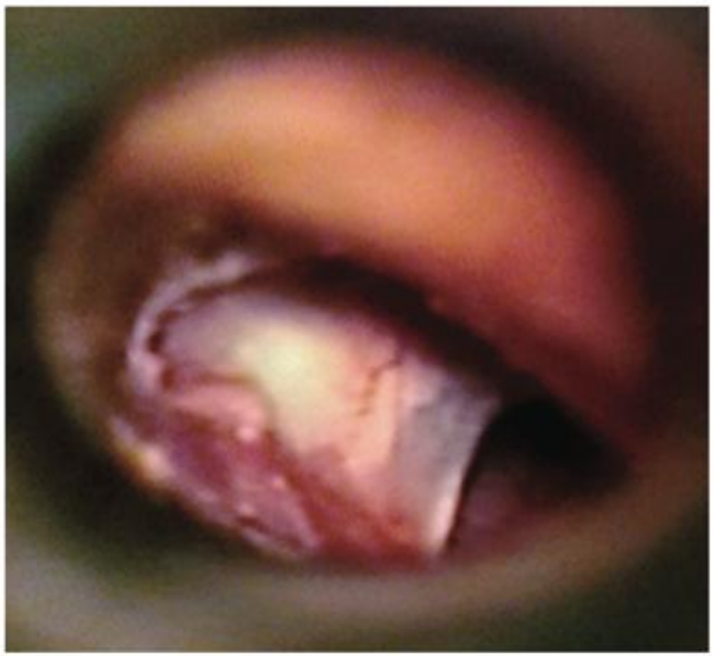
Complications
- 11 transient erythema
- No resorption during f/u

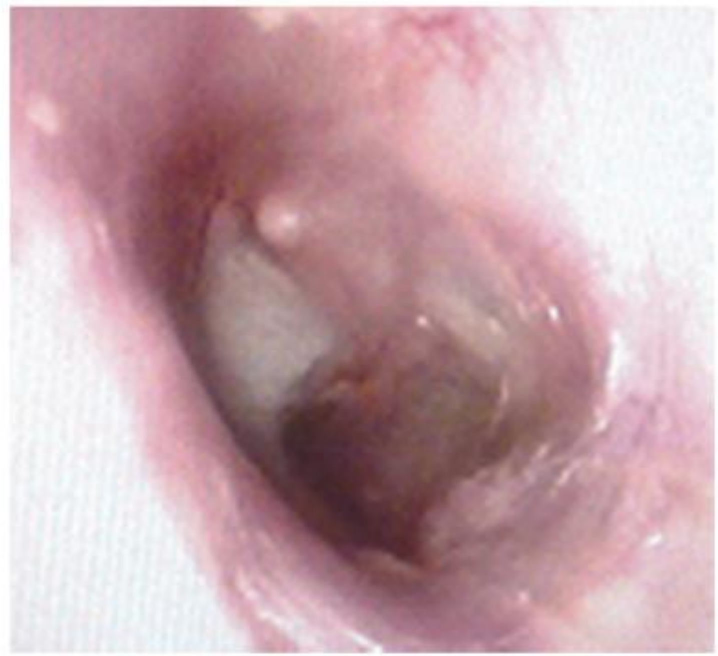
Mean follow up 15 months

No explantation of graft

PRP in Tympanic Membrane Perforations

- Evidence in Rats
  - Accelerates
- Case reports in humans with some success





J Laryngol Otol. 2009 May;123(5):482-7. doi: 10.1017/S0022215108003848. Epub 2008 Oct 28.

Platelet-rich plasma improves healing of tympanic membrane perforations: experimental study.

Erkilet E[1], Koyuncu M, Atmaca S, Yarim M.

PRP: jury's still out….

- Cochrane review 2012,
  - No difference in tx chronic wounds
    - "poor design of previous trials"

Other applications…

Bioengineered skin

Tissue Material	Tissue Layers	Living	Trade Name
Cultured keratinocytes autograft	Epidermal	Yes	Epicel (cultured epidermal autografts)
Acellular free-dried cadaveric skin allograft	Dermal	No	AlloDerm Regenerative Tissue Matrix
Bovine collagen/glycosaminoglycan/Silastic	Dermal	No	Integra Limit Uncertainty
Neonatal fibroblasts/polyglactin mesh allograft	Dermal	Yes	Dermagraft Human fibroblast-derived dermal substitute
Neonatal fribroblasts/keratinocytes collagen allograft	Composite	Yes	Apligraf add life to healing

Bioengineered skin: Apligraf

Biotechnol Bioeng. 1994 Apr 5;43(8):747-56.

Development of a bilayered living skin construct for clinical applications.

Wilkins LM[1], 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

Meir D. Hershcovitch, MD; David B. Hom, MD

ARCH FACIAL PLAST SURG/VOL 14 (NO. 6), NOV/DEC 2012 WWW.ARCHFACIAL.COM
391

Hyperbaric Oxygen

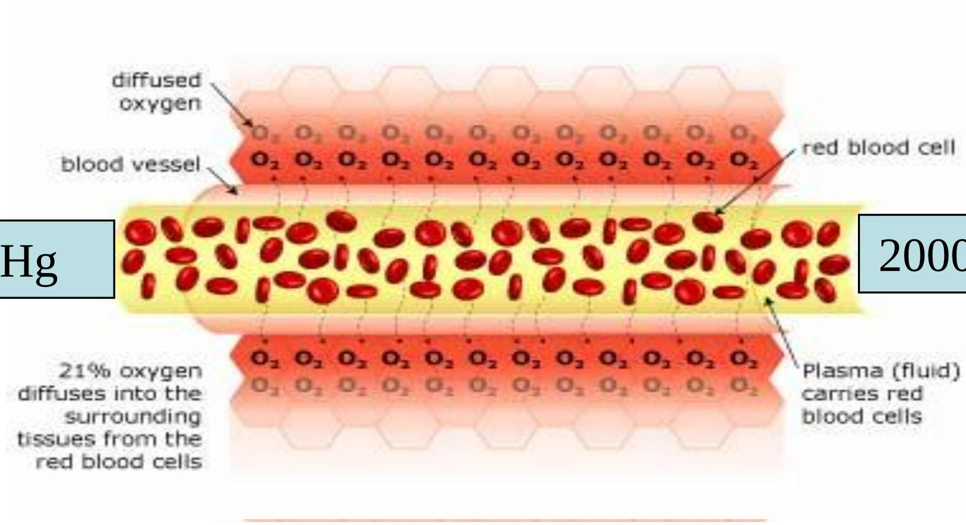
1 atm

Hyperbaric Oxygen

3 atm

55mm Hg

500mm Hg



100mm Hg

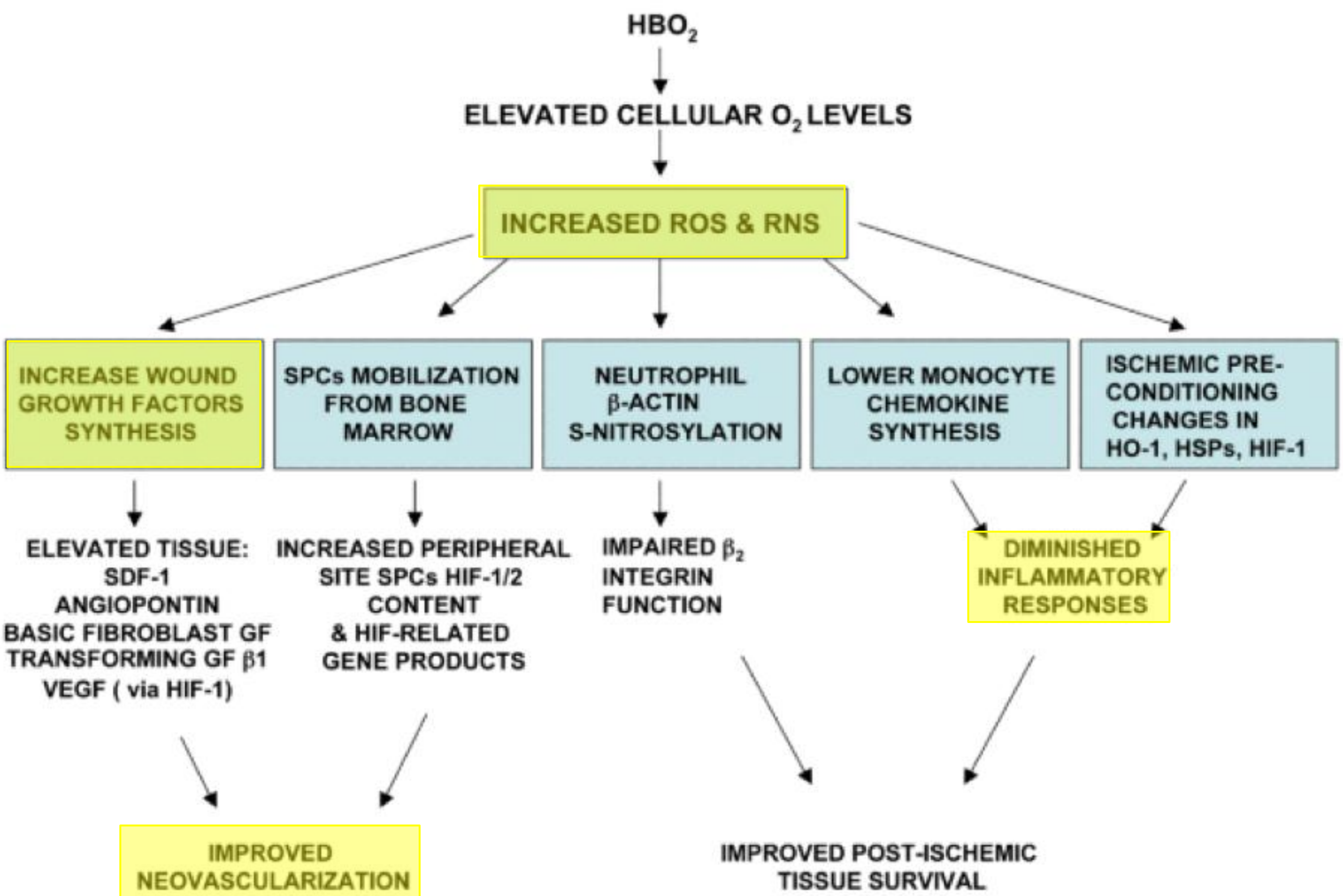
2000mm Hg

Plast Reconstr Surg. 2011 Jan;127 Suppl 1:131S-141S. doi: 10.1097/PRS.0b013e3181fbe2bf.

Hyperbaric oxygen: its mechanisms and efficacy.

Thom SR.

Hyperbaric Oxygen



Plast Reconstr Surg. 2011 Jan;127 Suppl 1:131S-141S. doi: 10.1097/PRS.0b013e3181fbe2bf.

Hyperbaric oxygen: its mechanisms and efficacy.

Thom SR.

[Intervention Review]

Hyperbaric oxygen therapy for late radiation tissue injury

Michael H Bennett[1], John Feldmeier[2], Neil Hampson[3], Robert Smee[4], Christopher Milross[5]

11 RCT, N = 669 → Significant Results:

2001-2011

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.

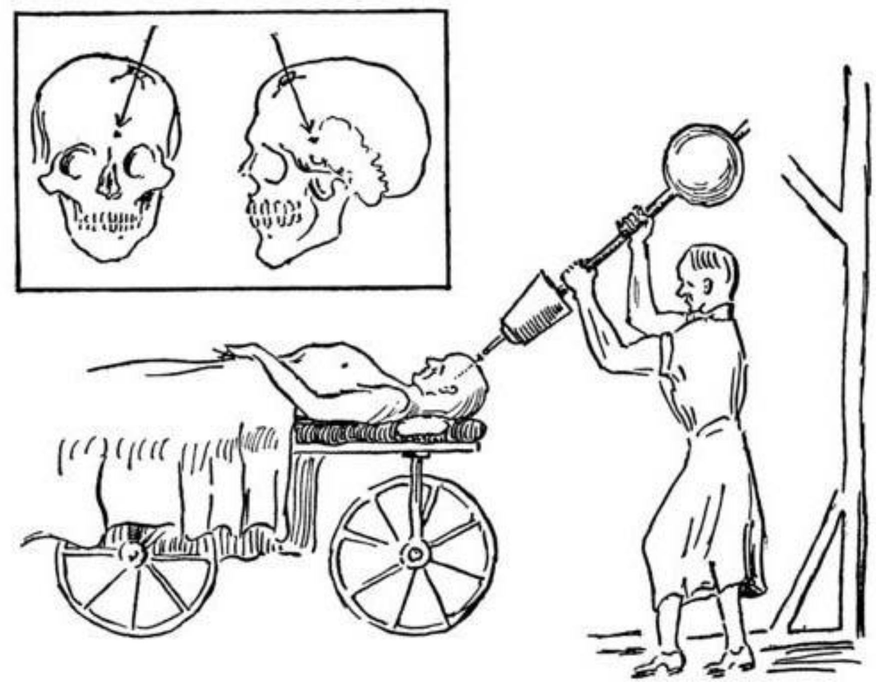
- Animal studies
  - Decreased distal necrosis
  - Free flaps, allowed prolonged ischemia
- Clinical studies
  - Cochrane review: 1 RCT STSG
  - "...high risk of bias"
  - "…more data needed"

Dr. Harvey Cushing

© WALTER WILLARD BOYD Walter Willard Boyd M.D.
P.B.B.H. 1928-1932

Doctor Cushing
Closing - 2000th Verified Brain Tumor
April 15, 1931.

Doctor Hoen



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U.S.News
HONOR ROLL 2013-14

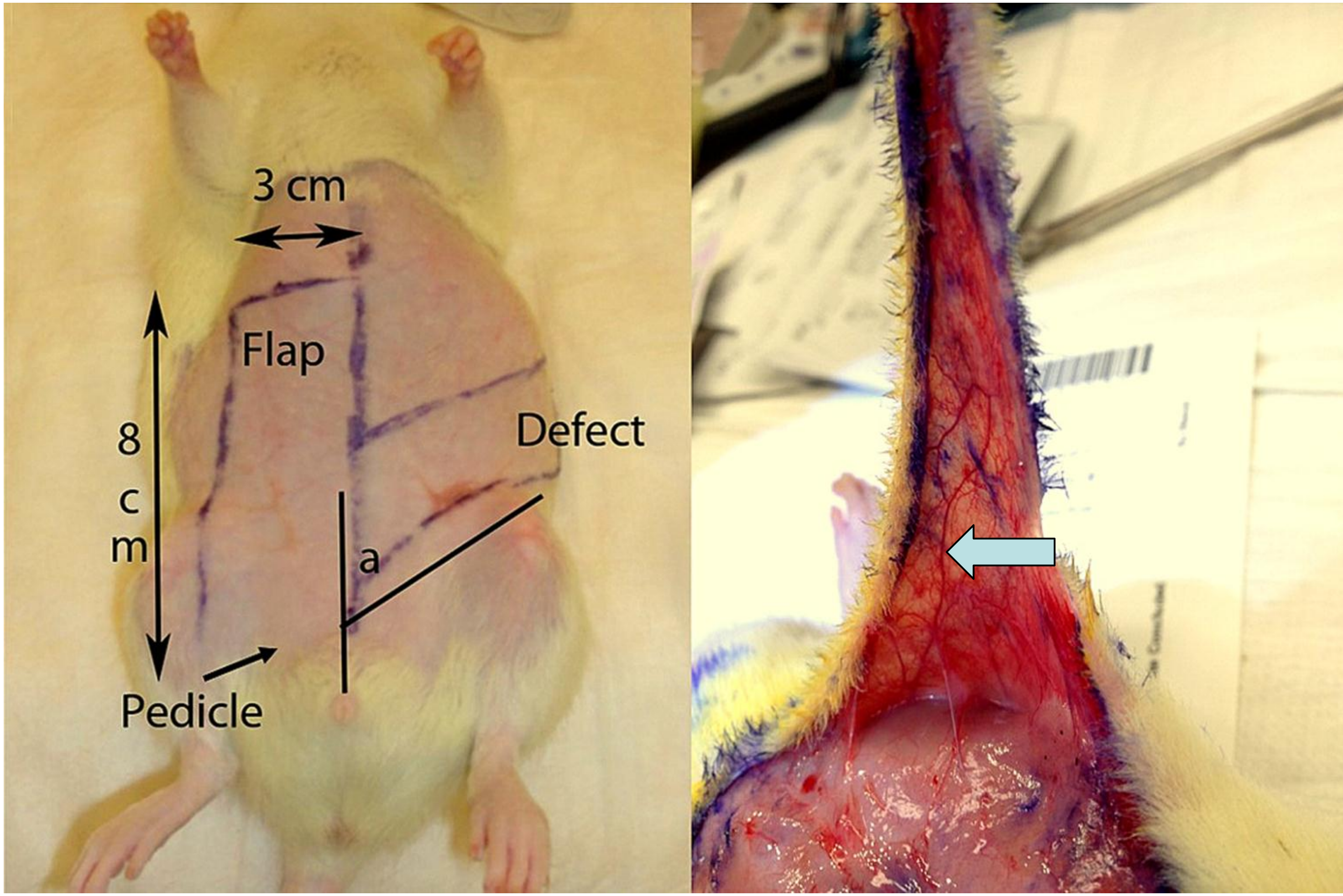
From
A Surgeon's Journal
HARVEY CUSHING

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



Pre Op

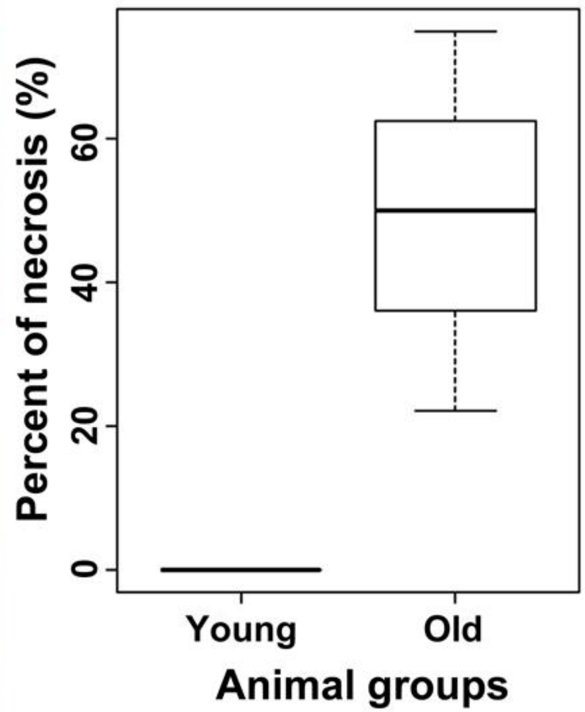

Protocol Number 734 F
Species Sprague Dawley
I.D.# 2583088 2
Date Entered 6/25/13
Dr. Pribitkin
Source Ch. River

Post Op

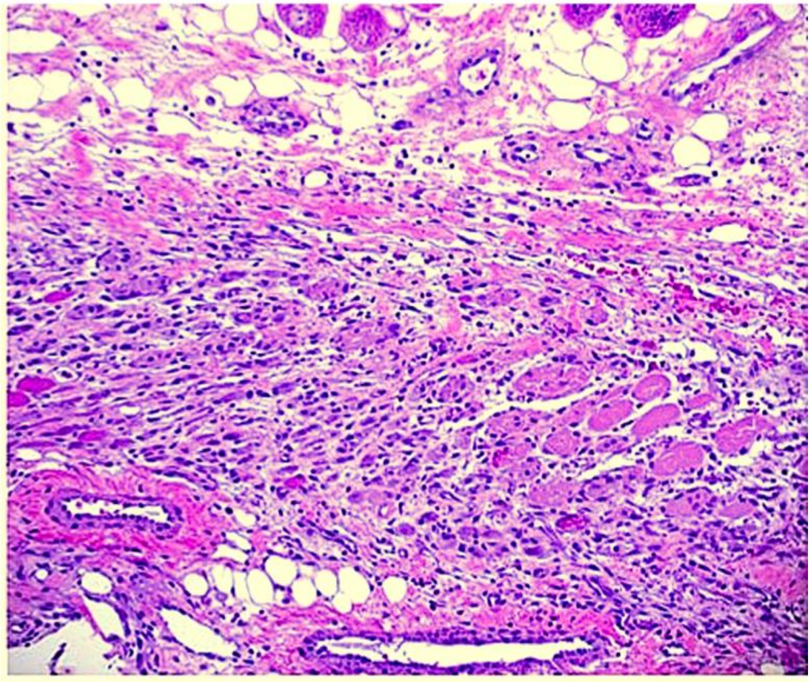

Protocol Number 734 F
Species Sprague Dawley
I.D.# 2583087
Dr. Pribitkin
Date Entered 6/25/13
Phone # 703-568-4200
Source Ch. River
Comments:

Old vs. Young

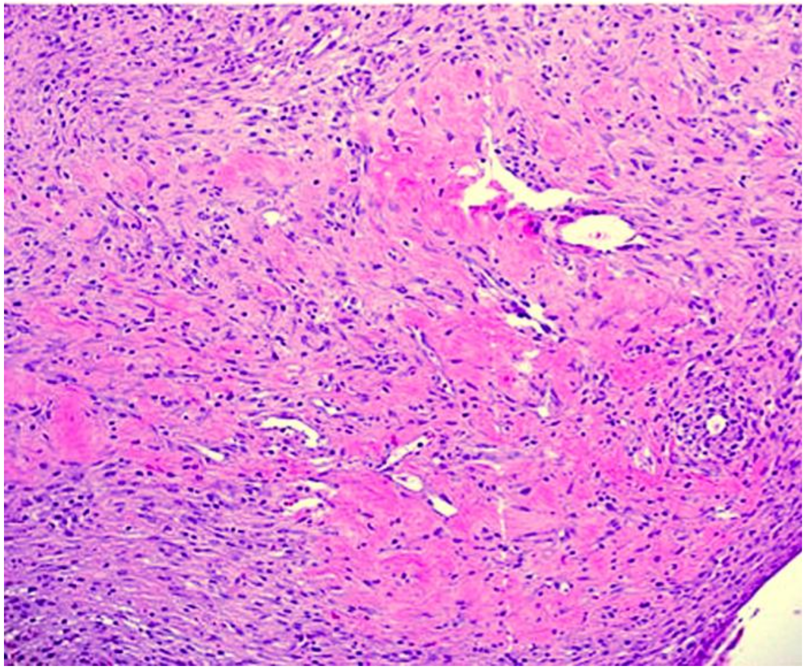
B.



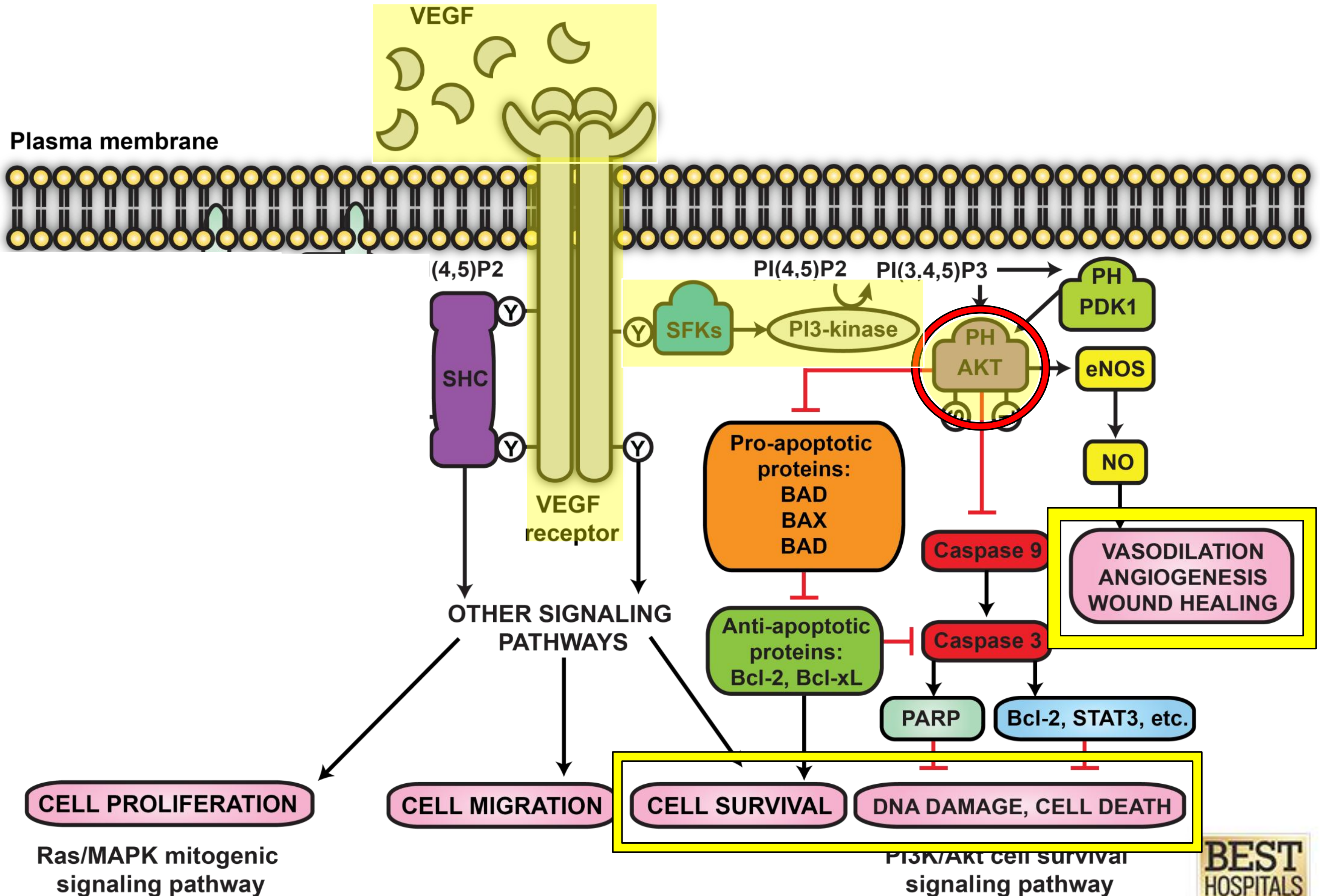
Histopathology



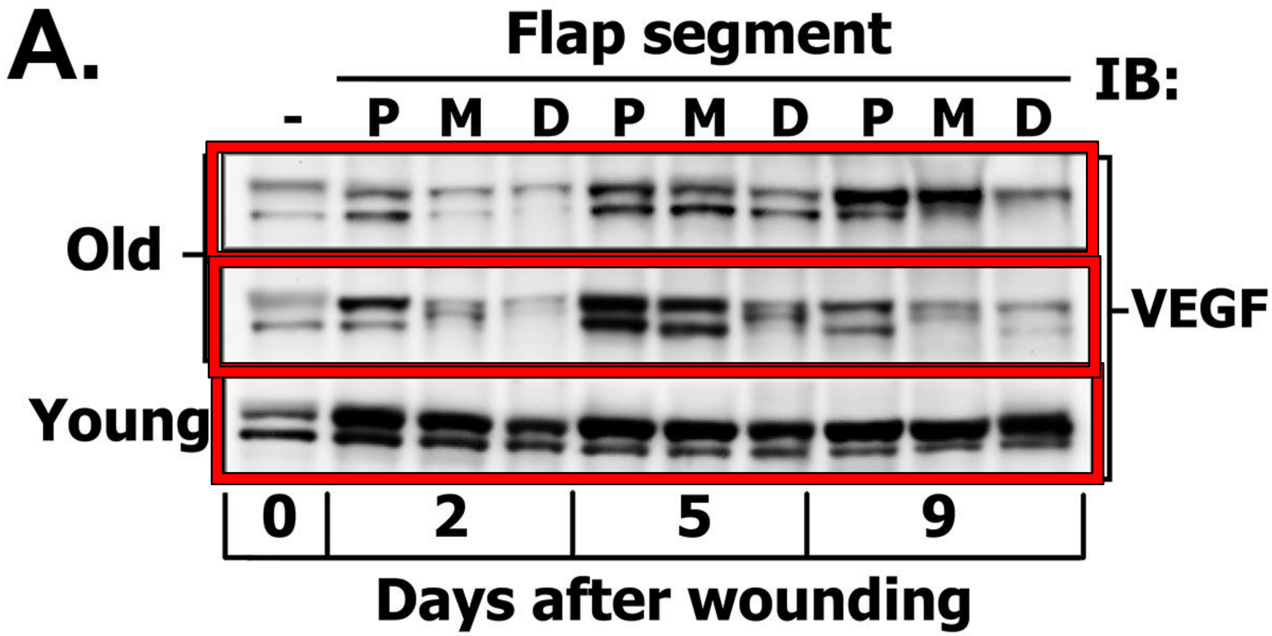
Young



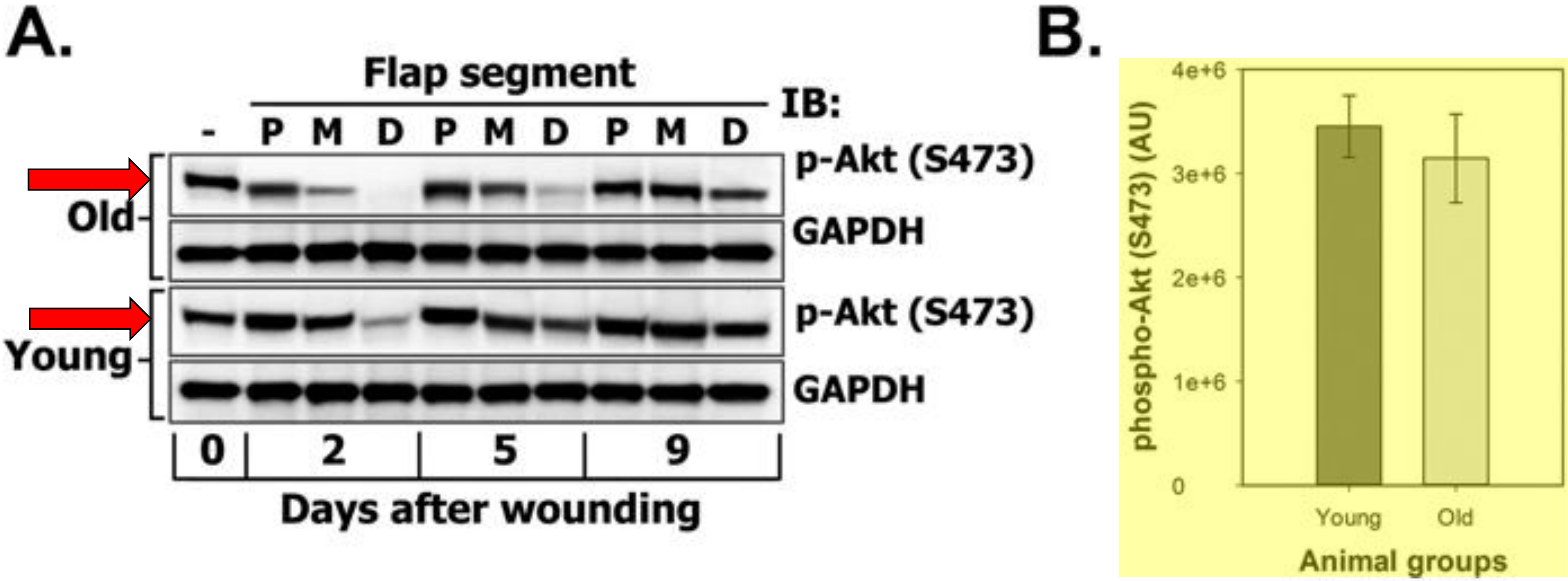
Old


VEGF
Plasma membrane
(4,5)P2
SHC
VEGF receptor
PI(4,5)P2
PI(3,4,5)P3
PH
PDK1
SFKs
PI3-kinase
PH
AKT
eNOS
NO
Pro-apoptotic proteins:
BAD
BAX
BAD
Caspase 9
VASODILATION
ANGIOGENESIS
WOUND HEALING
OTHER SIGNALING PATHWAYS
Anti-apoptotic proteins:
Bcl-2, Bcl-xL
Caspase 3
PARP
Bcl-2, STAT3, etc.
CELL PROLIFERATION
CELL MIGRATION
CELL SURVIVAL
DNA DAMAGE, CELL DEATH
Ras/MAPK mitogenic signaling pathway
PI3K/Akt cell survival signaling pathway

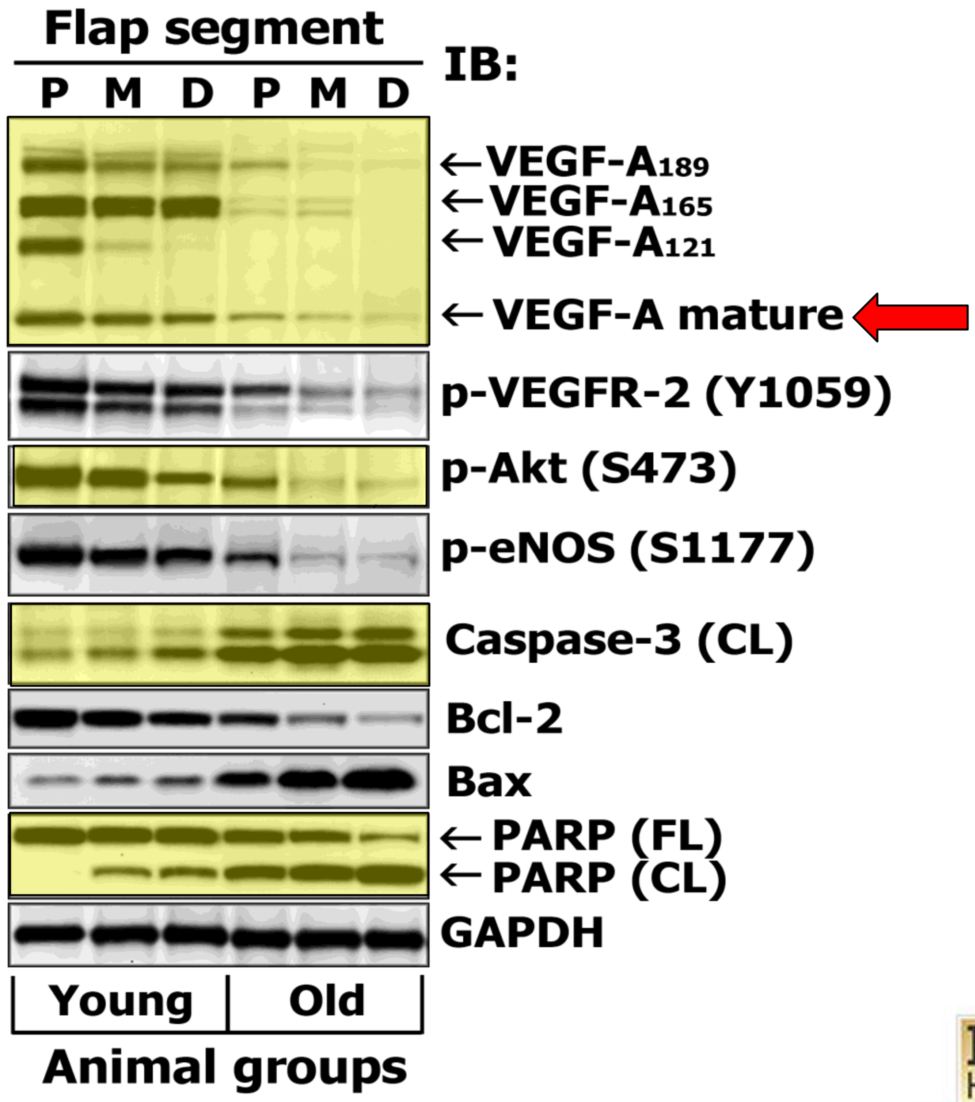
VEGF


A.
Flap segment
IB:
- P M D P M D P M D
Old
Young
VEGF
0 2 5 9
Days after wounding

Increasing Akt activation



Apoptosis



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US News
HONOR ROLL 2013-14

Jefferson.
University Hospital

Initial Conclusions What this really means….

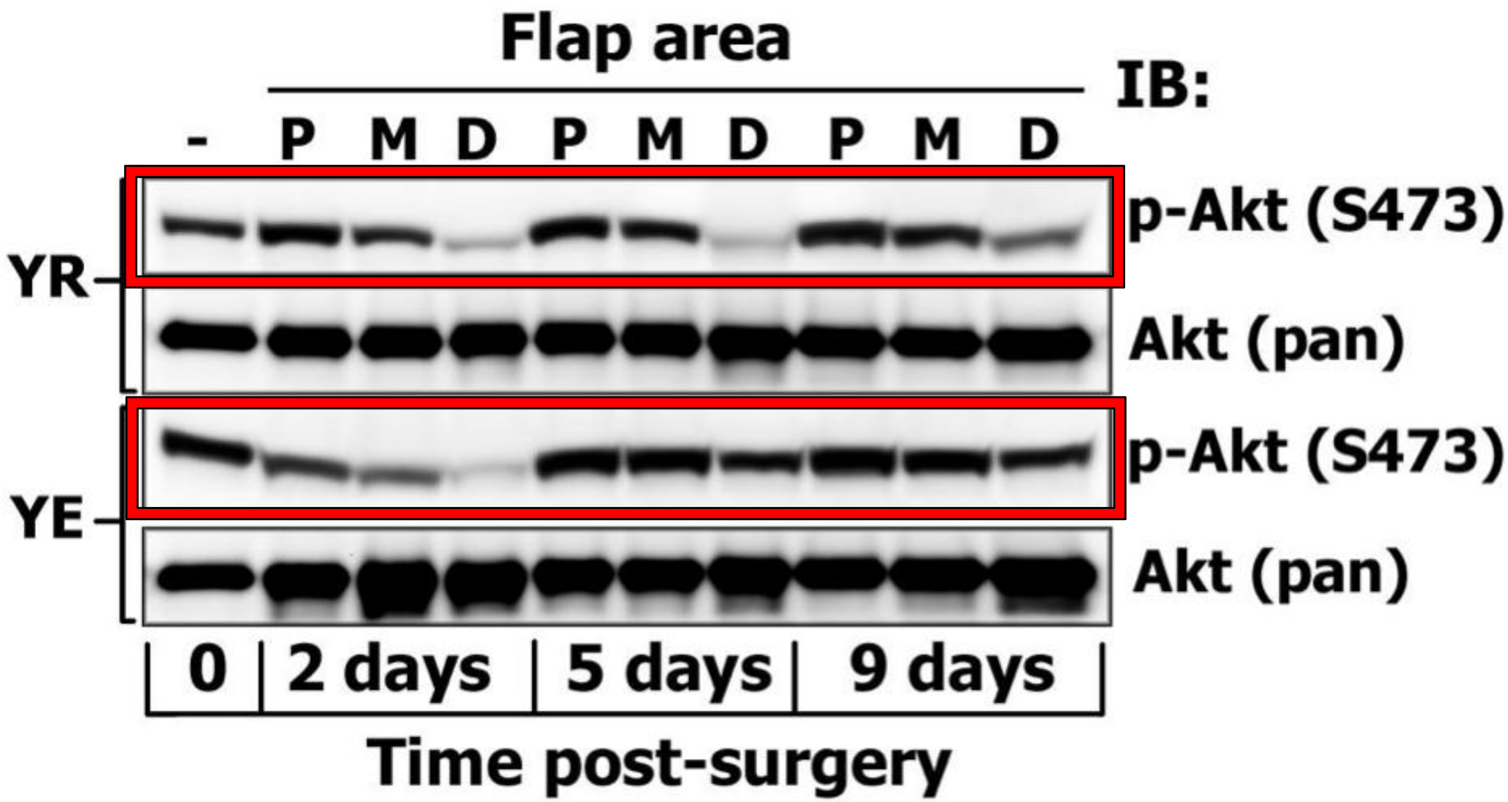
- Young vs. old
  - Increased VEGF
  - Increased Atk
  - Decreased apoptosis

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

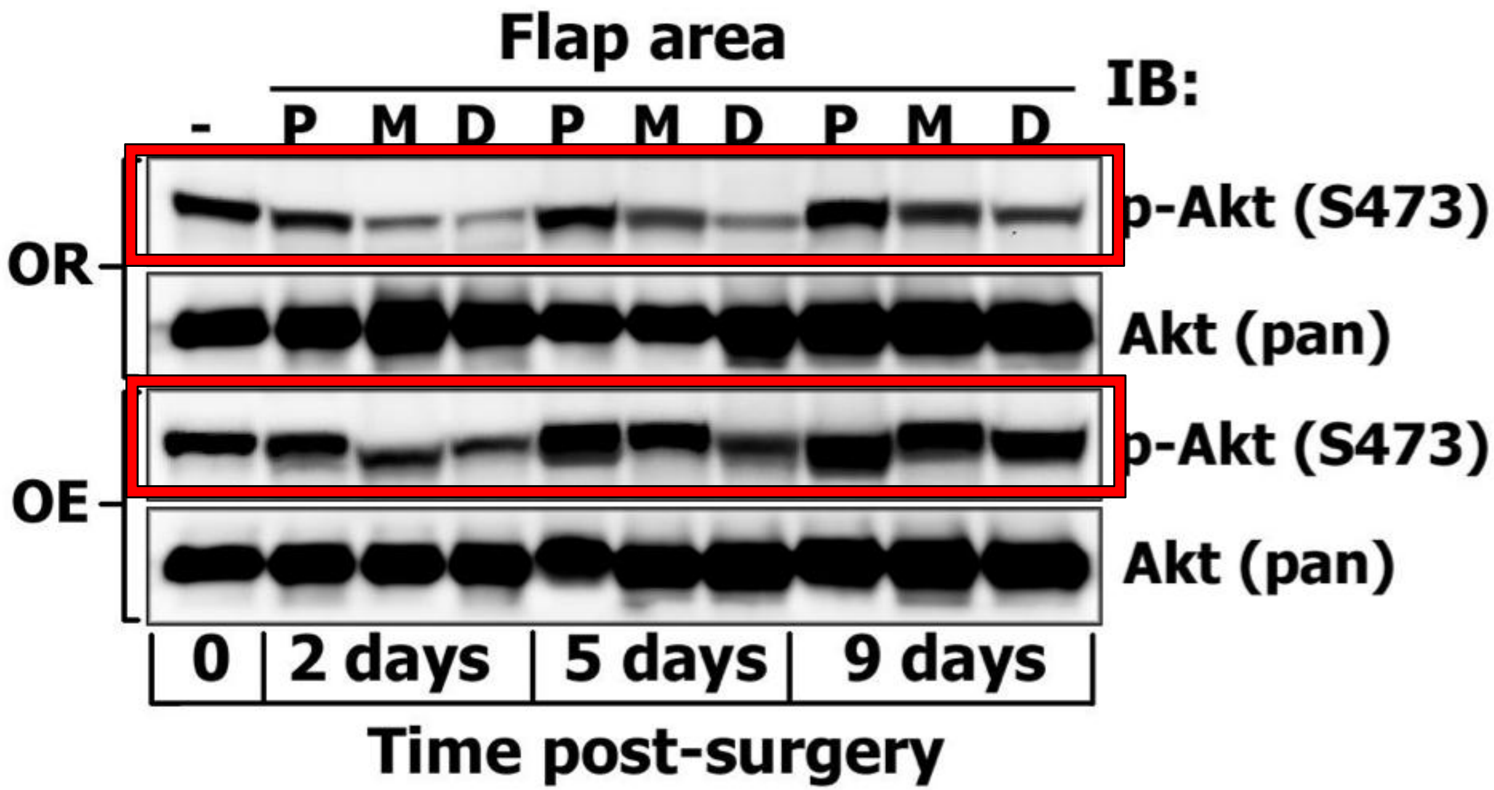
Exercise?

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

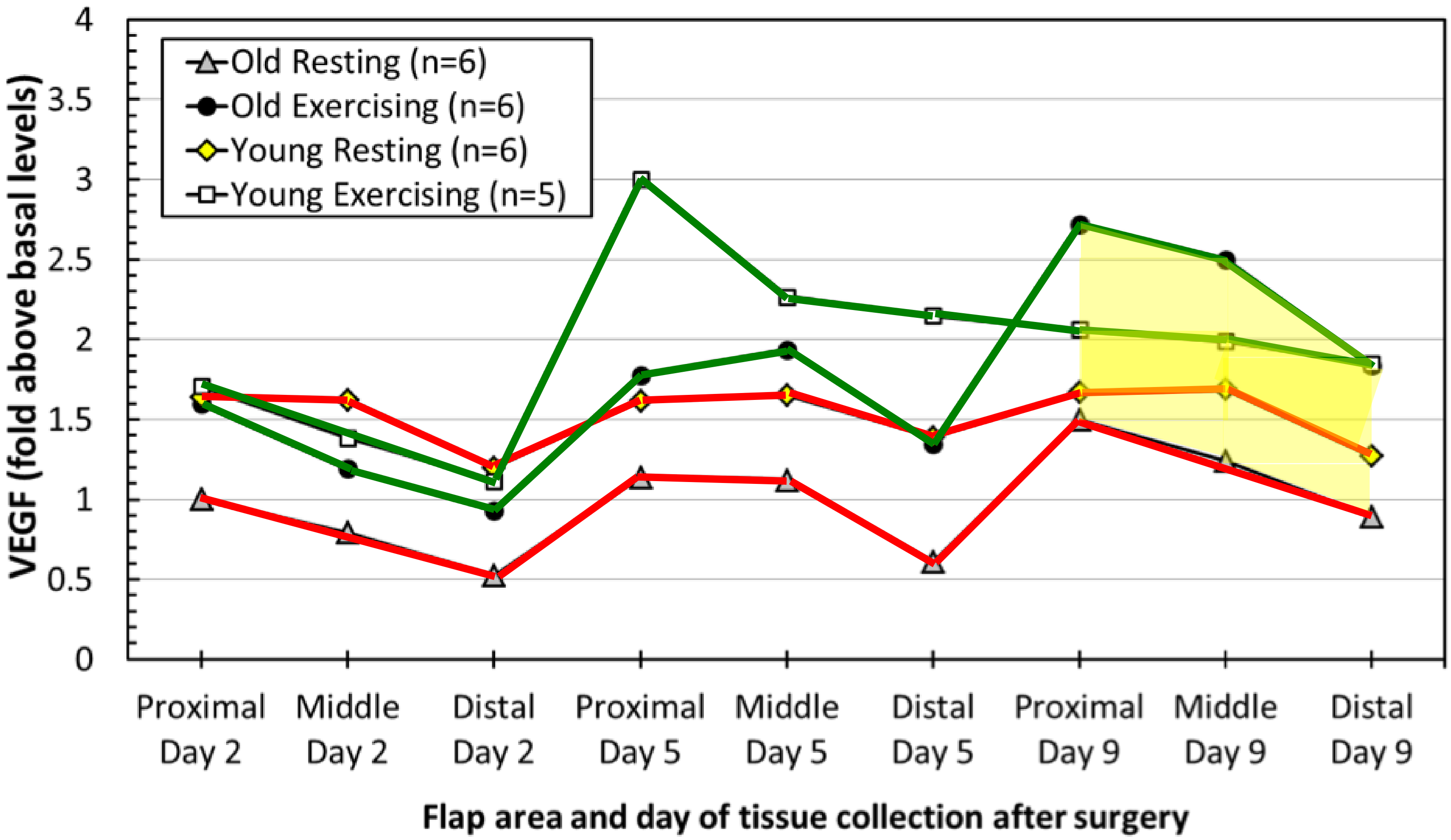
Effect of Exercise: Young Rats



Exercise Old Rats



VEGF



VEGF (fold above basal levels)
4
3.5
3
2.5
2
1.5
1
0.5
0
Old Resting (n=6)
Old Exercising (n=6)
Young Resting (n=6)
Young Exercising (n=5)
Proximal Day 2
Middle Day 2
Distal Day 2
Proximal Day 5
Middle Day 5
Distal Day 5
Proximal Day 9
Middle Day 9
Distal Day 9
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