Dermal Volumetric Rejuvenation Utilizing Autologous Platelet-Rich Plasma

Paris Meeting

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Background: Dr Jacques Otto MD

- 1981: Bachelor of Medicine & Surgery (Pretoria)
- 1986: Master of Family Medicine (Pretoria)
- 1988: Master of Medical Pharmacology (Pretoria)
- 1990: Diploma Forensic Medicine (SA College Med)
- 2000: M Phil Medical Law (Glasgow)

- 1990 – 1993: Medical Director Abbott Laboratories South Africa

- 2000 – 2009: Cosmetic Physician Harley Street (London) www.cosmedicate.com

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COSMEDICATE FOCUS : Non-surgical

- **Non- Surgical Facial Augmentation:**
  - Volumetric & Lines: Dermal fillers
  - Anti-wrinkle: Botulinum Toxin
- **Non-Surgical Facial & Body-contouring:**
  - Radio-Frequency skin tightening, lipolysis (Accent™ RF)
  - Body only Ultrasonic lipolysis (Ultrashape™)
- **Skin rejuvenation:**
  - Laser & IPL Broadband Light
  - Autologous Platelet-rich Plasma (A-PRP)
  - Dermasweep™ Dermabrasion & Infusion
  - Dermaroller™ micro-surgical needling
- **Vascular:**
  - Legs: sclerotherapy & laser & IPL
  - Face: laser & IPL
- **Acne & Rosacea:**
  - Aesthera™ • Accent™ RF• Dermasweep™
- **Research & Development:**
  - Lipolysis (CE Mark Ultrashape™),
  - Skin rejuvenation (A-PRP)

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Declaration

I was first introduced to autologous platelet-rich plasma by Dr Kubota of Japan in March 2006

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Fields of Application of a-PRP

RESEARCH & DEVELOPMENT	DENTAL MEDICINE	DERMATOLOGY INTERNAL MEDICINE GERONTOLOGY	SURGERY
Cell separation			Cardio-vascular surgery
Autologous cell culture	Dental extraction Dental implantation	Cutaneous reconstruction and transplantation	Abdominal surgery
Autologous stem cells culture			Maxillo-facial surgery
		Ulcer and chronic wound therapy (e.g. after radio therapy)	
Cell differential			Orthopaedic surgery
Tissue regeneration			Plastic & cosmetic surgery/dermatology
		Re-implantation of Autologous cells, extemporaneous or cultivated in-vitro	
Healing remodelling			Treatment of severe burns

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Autologous Platet-richPlasma

- What is plasma?
  - Fluid component of a person's blood
  - Contains platelets, white blood cells, stem cells, electrolytes, enzymes, hormones, nutrients, anti-bodies, glucose, proteins, lipids & albumin (powerful anti-oxidant), etc.
- Why autologous plasma?
  - Autologous means person's own (self donation) and not donated from another person or from animal origin
- How can plasma be obtained easily?
  - Venous blood sample is obtained from patient's fore-arm
  - Centrifugation separates plasma & platelets & stem cells from red blood cells

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What is Autologous Platelet Rich Plasma (A-PRP)?

- A-Platelet rich plasma is a concentration of human platelets in a small volume of plasma measured as 1,000,000 platelets per mm^3 or 2-6 times the native concentration of whole blood at a pH of 6.5 - 6.7 (whole blood pH = 7.0 - 7.2)
- Also referred to as autologous platelet gel, plasma-rich growth factors (PRGFs) or autologous platelet concentrate
- PRP is also a concentration of the 7 fundamental protein growth factors that have been proved to be actively secreted by platelets to initiate all wound healing
- PRP includes 3 proteins in blood known to act as cell adhesion molecules: fibrin, fibronectin & vitronectin

Some Growth Factors Acting on “Healing Cascade”

Factor	Name	Principal Source	Effects
PDGF aa PDGF bb PDGF ab	Platelet derived growth factors	Activated thrombocytes	Mitogenes of mesenchymal stem cells promote the synthesis of the extracellular matrix
TGF- alpha TGF- beta	Transforming Growth Factors	Activated thrombocytes	Stimulation of DNA synthesis, proliferation of various types of cells. Favours the synthesis of collagen
IGF- I IGF- II	Insulin-like Growth Factors	Activated thrombocytes	Stimulates the proliferation and differentiation of osteoblasts
EGF	Epidermal Growth Factor	Activated thrombocytes	Stimulates proliferation and differentiation of epidermis cells, co-stimulating angiogenesis
VEGF	Vascular Endothelial Growth Factor	Leucosytes & Endothelial cells	Stimulate angiogenesis & chemo-attraction of osteoblasts

In addition the activated thrombocytes have onto their surface a multitude of signalisation molecules eg. CD9, CD-W17, CD31, CD41, CD42a-d, CD51, CD-W60, CD61, CD62P, CD63

How are Platelets Activated?

- Dermal collagen & exposed endothelial collagen
- Arachidonic Acid (inflammation pathway)
- Thromboxane A2 (inflammation pathway)
- ADP
- Thrombin
- Substrate bound ligands of Glycoprotein II a / III b
- Vasopressin
- Adrenaline
- $CaCl_2$
- Controlled Heat (Radio-frequency)
- Vibration (?) via Vortex device
- Non-thermal dielectric barrier discharge plasma treatment

Key Mediators in Platelet Adhesion, Activation and Aggregation

1. Ferguson JJ. *The Physiology of Normal Platelet Function*. In: Ferguson JJ, Chronos N, Harrington RA (Eds). *Antiplatelet Therapy in Clinical Practice*. London: Martin Dunitz; 2000: pp.15–35.

2003 Dia-Präsentation von Sanofi-Synthelabo - Bristol-Myers Squibb aus dem Jahr 2002 ©

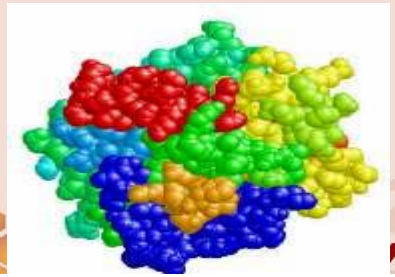
Plasma forms a biological 'scaffold' in-vivo

Via the action of the thrombin…

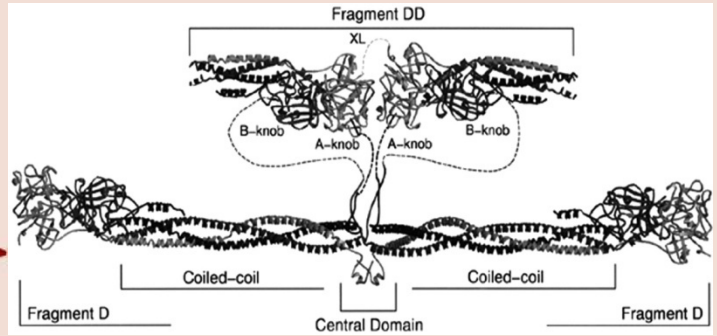
Fibrinogen is transformed to fibrin strands

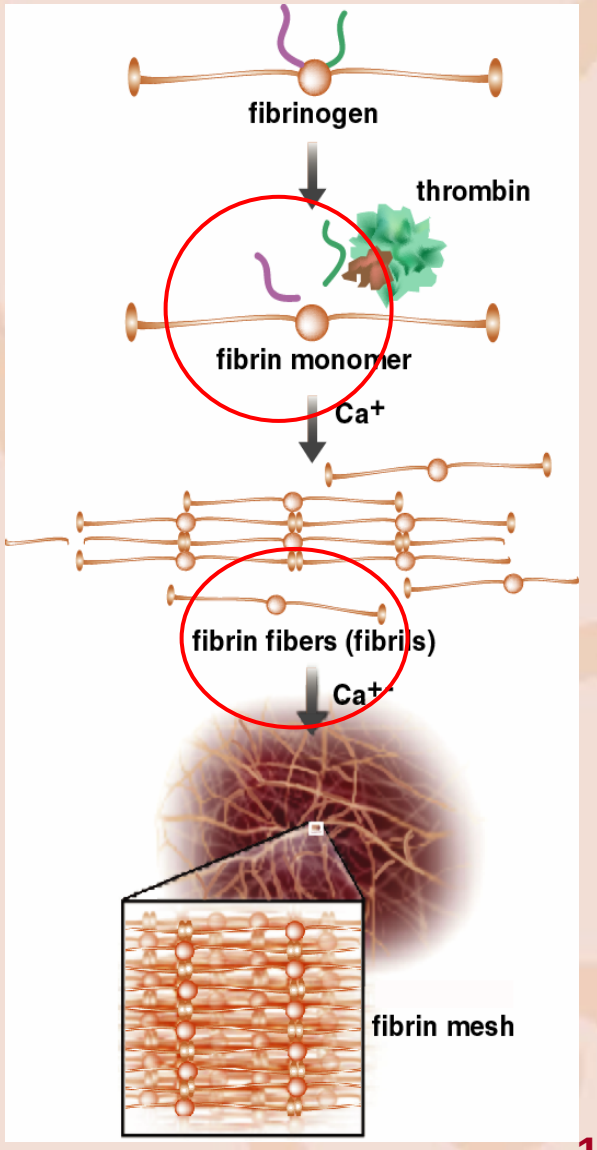
3-D polymeric structure is formed through the binding of fibrin monomers

'Imprisonment' of leucocytes and platelets in the polymeric structure (covalent links): clot formation



3D Computer Model of Human Thrombin



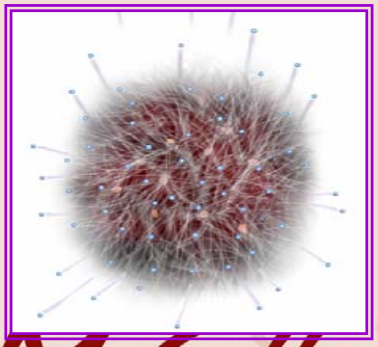


Fibrinogen: Dr Doolittle

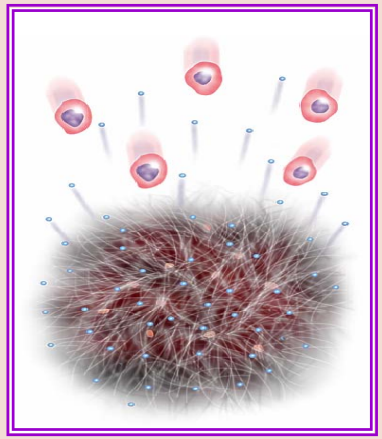
The 5 Major Steps In The Platelet Activation Process

1. Formation of tri-dimensional mesh (fibrin strand)or matrix….

2. Release of growth factors by the thrombocytes and leukocytes….

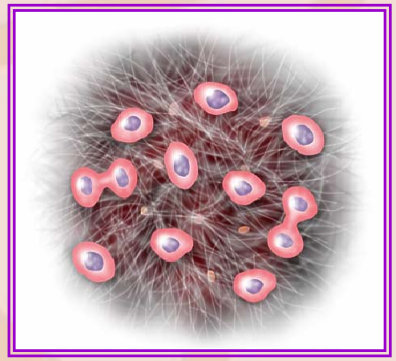


3. Chemo-attraction or migration of macrophages and stem cells…

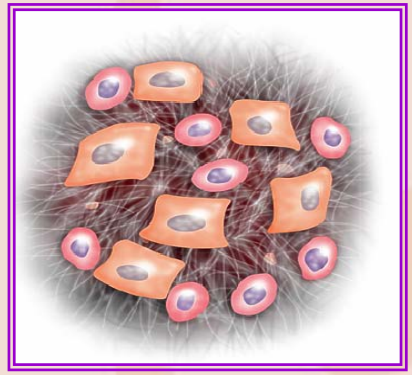


(In addition ECM like fibronection, vitronecton, thrombospondin…)

4. Stem cells proliferation & mitosis…

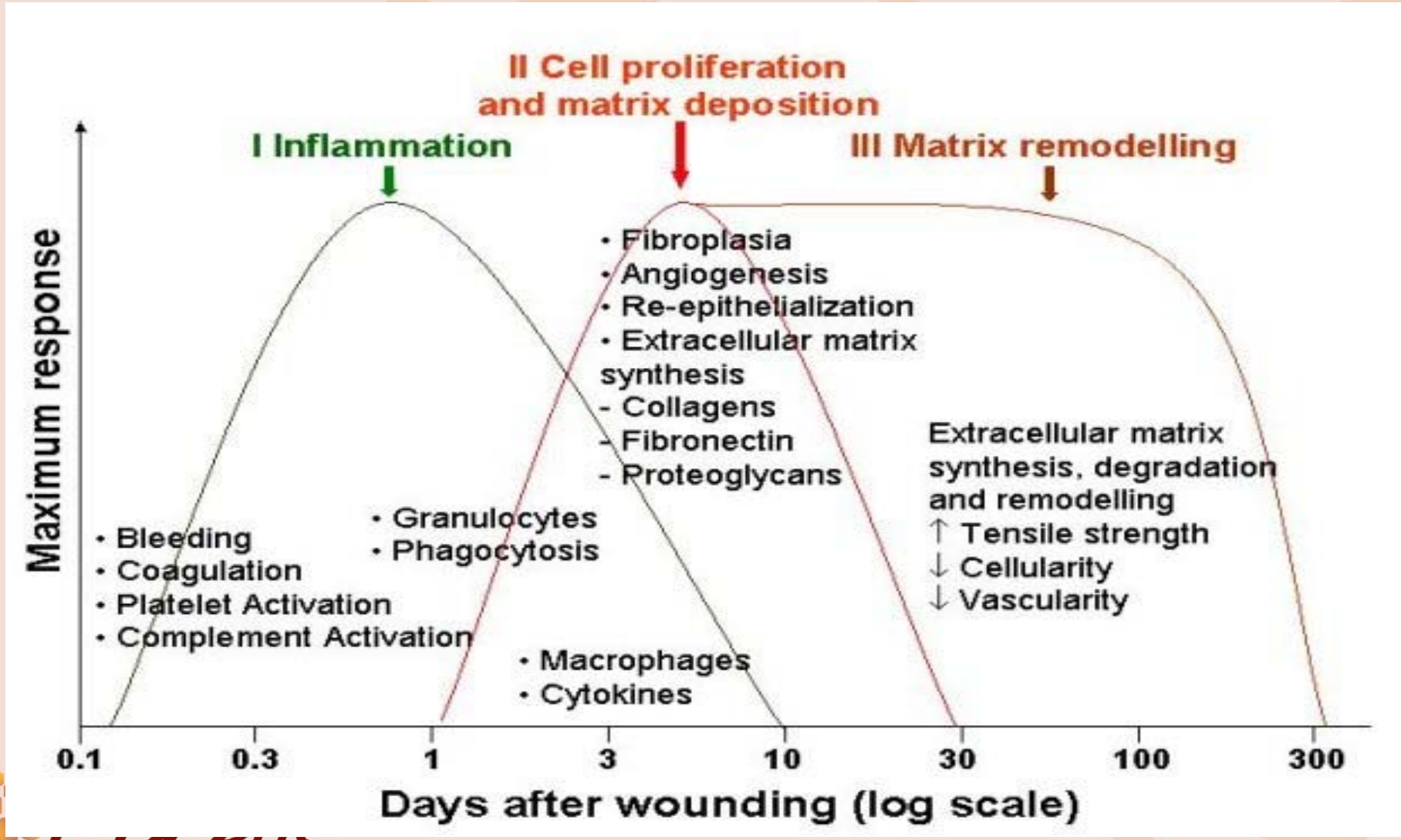


5. Stem cells differentiation…

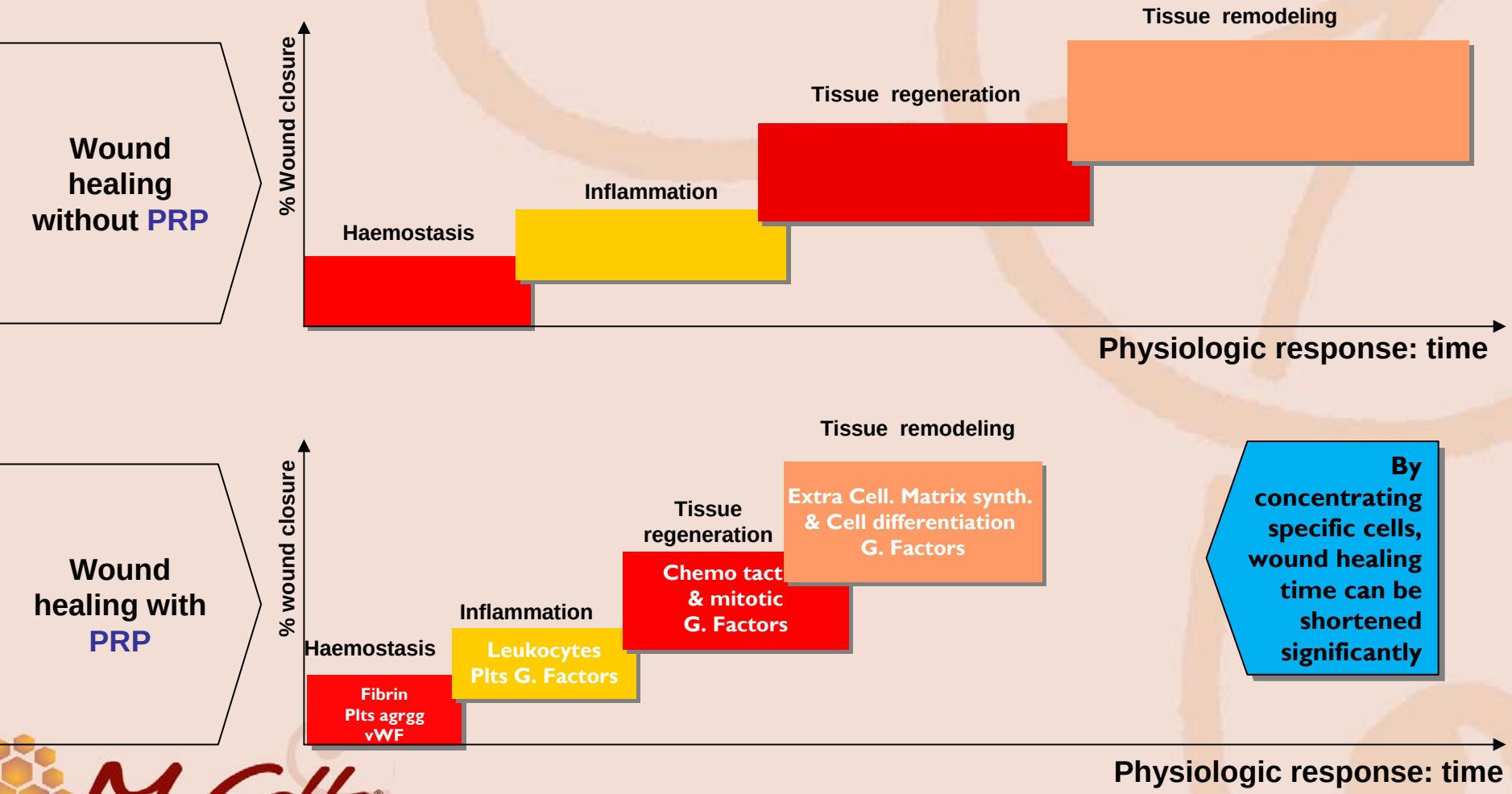


*Platelets & Megacaryocytes vol.2 Dr J.Gibbins, M. Mahaut-Smith

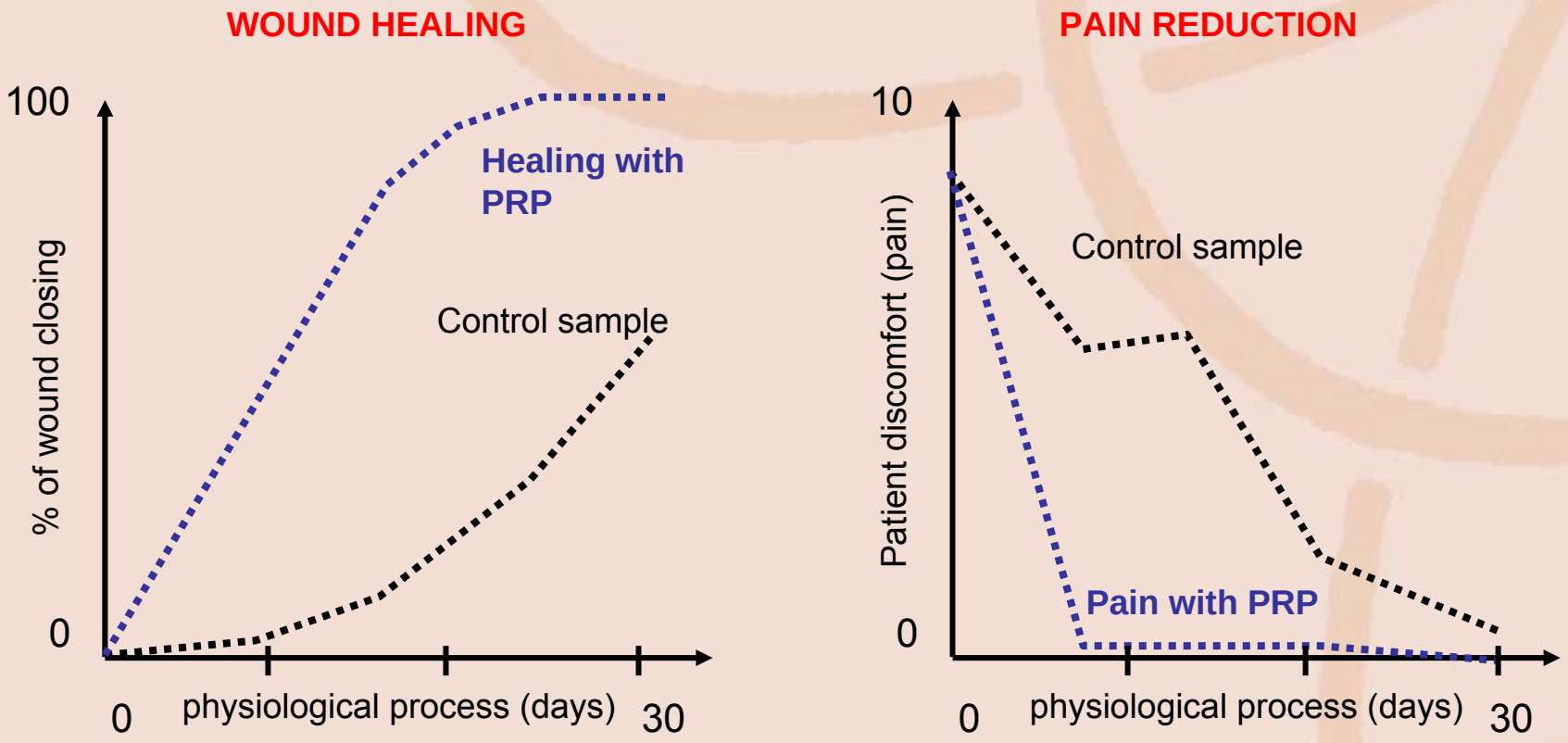
Timelines in Wound Healing



Benefits of a-PRP reported in the “Healing Cascade”



Visible effect in time of Healing and Discomfort (randomized study USA)



Journal of Oral & Maxillofacial Surgery, 2000; 58:45 Marx, Monteleone, Ghurani, Dr. Robert Marx, University of Miami

Advantages of a-Plasma

- **Tissue regeneration & rejuvenation**: neo-collagenesis (TGFα & β), neo-vascularisation (EGF & VEGF), & extracellular matrix formation (PDGFαα & ββ & αβ) – NB: growth factors in genetically pre-determined ratio!
- **Bio-glue (fibrin glue)**: haemostasis & tissue adhesion in skin flaps, bone grafts, trauma intra-surgery and post-surgery
- **Safety:** non-allergenic & free from concerns over transmissible diseases e.g. HIV, Hepatitis B & C, CJD, etc.
- **Autologous**: no risk of rejection reaction
- **Wound healing time**: increased
- **Physiological 'anti-biotic' :** anti-bodies & WBC's & proteolytic enzymes
- **Plasma includes:** hormones, biotransformed vitamins & other nutrients
- **Tissue engineering:** in-vitro autologous tissue culture-medium.
- **Ease of use**: dermal & hypodermal injections
- **Convenience:** Plasma harvesting performed in doctor's rooms (no external laboratory required)
- **Cost effectiveness:** 1 Plasma kit (2 tubes) delivers 12 ml Plasma

Contra - Indications

- Platelet Dysfunction Syndrome
- Critical Thrombocytopenia
- Hypofibrinogenaemia
- Haemodynamic Instability
- Auto-immune disease
- Malignancy
- Sepsis
- Acute & Chronic Infections
- Chronic Liver Pathology
- Anti-coagulation therapy
- Pregnancy (for cosmetic indications)

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Potential Complications (applicable to all dermal fillers)

Intra-vascular injection (thrombus/embolus)

-Venous

-Arterial

Nerve trauma (needle)

Secondary infection

Beware of the peri-ocular area (eyelids)!

A-PRP Indications

1. Skin rejuvenation:
   - injection (intradermis)
   - mesotherapy (intradermis)
   - topical plasma & Dermaroller (micro-needling) intradermis
2. Fine lines & wrinkles: ditto
3. Volumetric 'filling' :
   - large volume injection of A-PRP intradermis and hypodermis of the tear troughs, eyelids, naso-labial folds, marionette lines, peri-oral areas, cheeks, forehead, glabella, neck & back of hands
   - A-PRP mixed with fillers such as hyaluronic acid and calciumhydroxyl-appatite (Radiesse) = 'bio-active' filler
4. Acne scarring:
   - subscision injections & topical A-PRP & Dermaroller
5. Cellulite?
6. Striae (stretch marks)?

SKIN TREATMENT ACTION LEVELS

MULTIDISCIPLINARY PROGRAM FOR THE REJUVENATION OF THE FACE

DERMO COSMETICIS

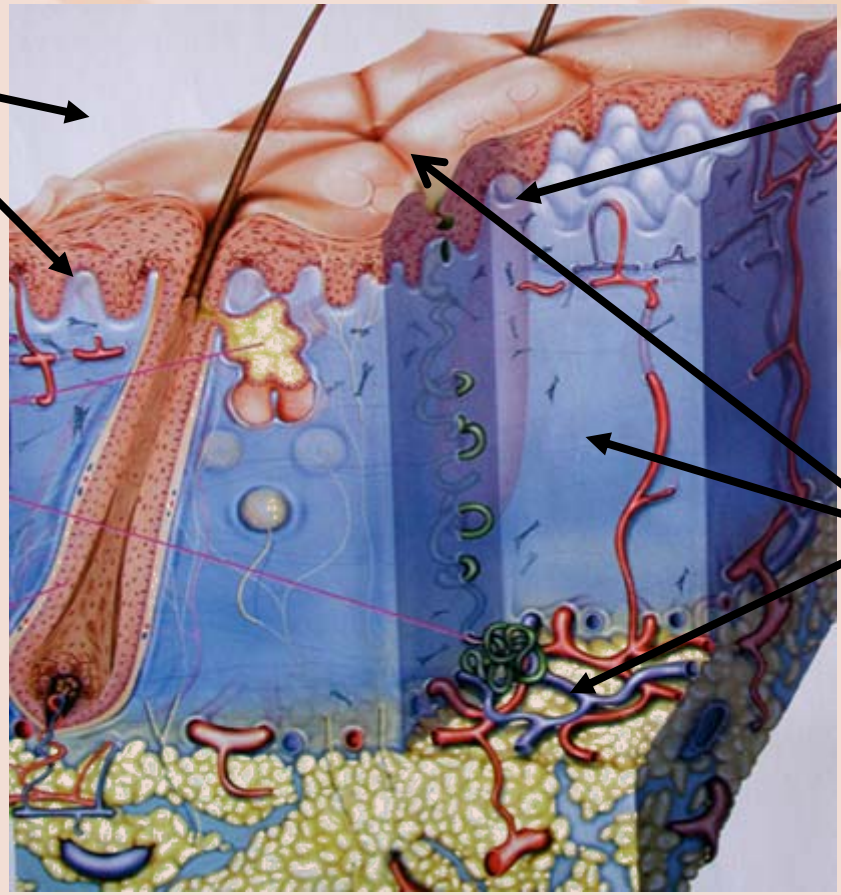
PEELS

Renewal of the corneal layer

Germinative layer Stimulation

Dermis Stimulation

Hyperpigmentation removal



FILLERS

Wrinkles & volumes correction

BIO STIMULATION MyCells

Architectural skin reconstruction/ reorganization

Pre-Treatment Patient Preparation & Combination Therapy

- High Dose Oral Vitamin C (1,000mg+) daily for 7 days pre-treatment & post-treatment
  - Enhances wound healing
- Oral Vitamin A daily for 7 days pre- & post-treatment
- Radio-Frequency
  - Immediately after treatment = platelet activation
  - Collagen fibre contraction (immediate)
  - Fibroblast induced neo-collagenesis (delayed)
- Dermaroller & Topical A-PRP
  - micro-surgical needling
  - Induces growth factor release
- Topical Vitamin A
- Topical Vitamin C

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A-PRP Harvesting

Vena puncture

Centrifugation @ 3,500 r.p.m. x 7 min

6ml A-PRP per tube

Preparation of A-PRP

STEP 1: Draw venous blood (vacuum allows 10 ml max. per tube)

STEP 2: Centrifuge @ 3,500 r.p.m. x 7 minutes

STEP 3: Tube plasma yield = 6 ml

STEP 4: Remove 3ml (50%) of plasma from top of tube (Platelet Poor Plasma) if enrichment (PRP) is required = doubled platelet concentration

STEP 5: Re-suspend platelets & WBC's in remaining 3 ml of plasma with vortex device and allow to stand for 5 minutes (growth factor release by platelets)

STEP 6: Inject A-PRP intra-dermal or sub-dermal

STEP 7: Fibrin matrix formation after 10 – 15 minutes

MyCells® Tube vs Vacutainer® Tube

A-PRP-tube (MyCells®)

- Developed for therapeutic application
- Anti-coagulant CD-A for optimal preservation of the structure & functional integrities of leucosytes & thrombocytes
- Gel separator in ACR tube allows for optimal yield of thrombocytes (> 90%)
- ISO 13485: 2003 tested for good tolerance & absence of allergenic & mutagenic effects
- CE Mark Class IIa (medical device) ***for Re-injection!***

Vacutainer®

- Therapeutic application illegal
- Anti-coagulant EDTA damages the structure and function of cells, especially thrombocytes
- If present, then the gel separator acts differently to the ACR gel
- Can contain traces of intolerant clotting activator and allergenic substances

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MyCells® Tube vs Regenlab® Tube

MyCells®

- Plasma yield = 6 ml
- Clear separation of plasma from red blood cells
- Optimal yield of thrombocytes (> 90%)
- ISO 13485: 2003 tested for good tolerance & absence of allergenic & mutagenic effects
- CE Mark Class IIa (medical device) ***for Re-injection!***

Regenlab®

- Plasma yield = 4.5 ml
- Incomplete red blood cell separarion from plasma
- Platelet yield 60 – 70%
- ISO 1250: 2003 tested for good tolerance & absence of allergenic & mutagenic effects
- CE Mark Class IIa (medical device)

OTTO Plasma Therapy Technique (1)

- Venous blood centrifuged @ 3,500 rpm x 7 minutes = 6 ml plasma yield
- Platelet activation: 3 ml plasma + 0.3 ml of 2% lidocaine & adrenaline 1:100, 000 (ratio 1:10). 3 ml of PPP not discarded because it also contains platelets!
  - No addition of $CaCl_2$ (causes tissue irritation)
  - Adrenaline activates platelets (not effective in 20% cases) Vasopressin?
  - Lidocaine is anaesthetic & anti-bacterial (MRSA, Staph , Strep, E.coli Pseudomonas, etc.)
- Vortex plasma x 30 seconds to liberate platelets, white blood cells and stem cells from gel
- Injection of A-PRP into dermis and hypodermis of eyelids, naso-labial folds, lips, cheeks, marionette lines & neck causes:
  - tissue expansion (triggers wound healing cascade)
  - needle-trauma (triggers wound healing cascade
  - stem cell and activated platelet deposits leading to enhanced wound healing
- Dermis injections stimulate fibroblasts & keratinocytes = skin tightening
- Hypodermis injections stimulate pre-adipocytes = fat cell volumetric effect

OTTO Plasma Therapy Technique (2)

- Accent Radio-frequency: bipolar immediately after injections
  - Controlled dermal heating to max 40° C (max 15 min)
  - Stimulation of micro-circulation
  - ADP-induced platelet aggregation & clot retraction.
- Dermaroller f
  - 250 micro-needle puncture wounds per cm2
  - Causes growth factor release, fibroblast & stem cell activation
  - Stimulation of micro-circulation
  - Apply topical A-PRP before Dermaroller
  - Dermaroller to full face and neck until pinpoint bleeding occurs
  - Re-apply A-PRP after Dermaroller: thrombin from pin-point bleeding activates platelets and initiates fibrin strands (plasma' setting')

OTTO Plasma Therapy Technique (3)

Presence of Progenitor $CD34^+$ Bone Marrow Stem Cells in MyCells A-PRP

- Neocell Laboratories in Cape Town 2008
- 10 ml Venous blood sample:

  1.6×10^9 $CD34^+$ stem cells
- 6 ml post centrifugation A-PRP:

  1.2×10^9 $CD34^+$ cells = 80% yield!

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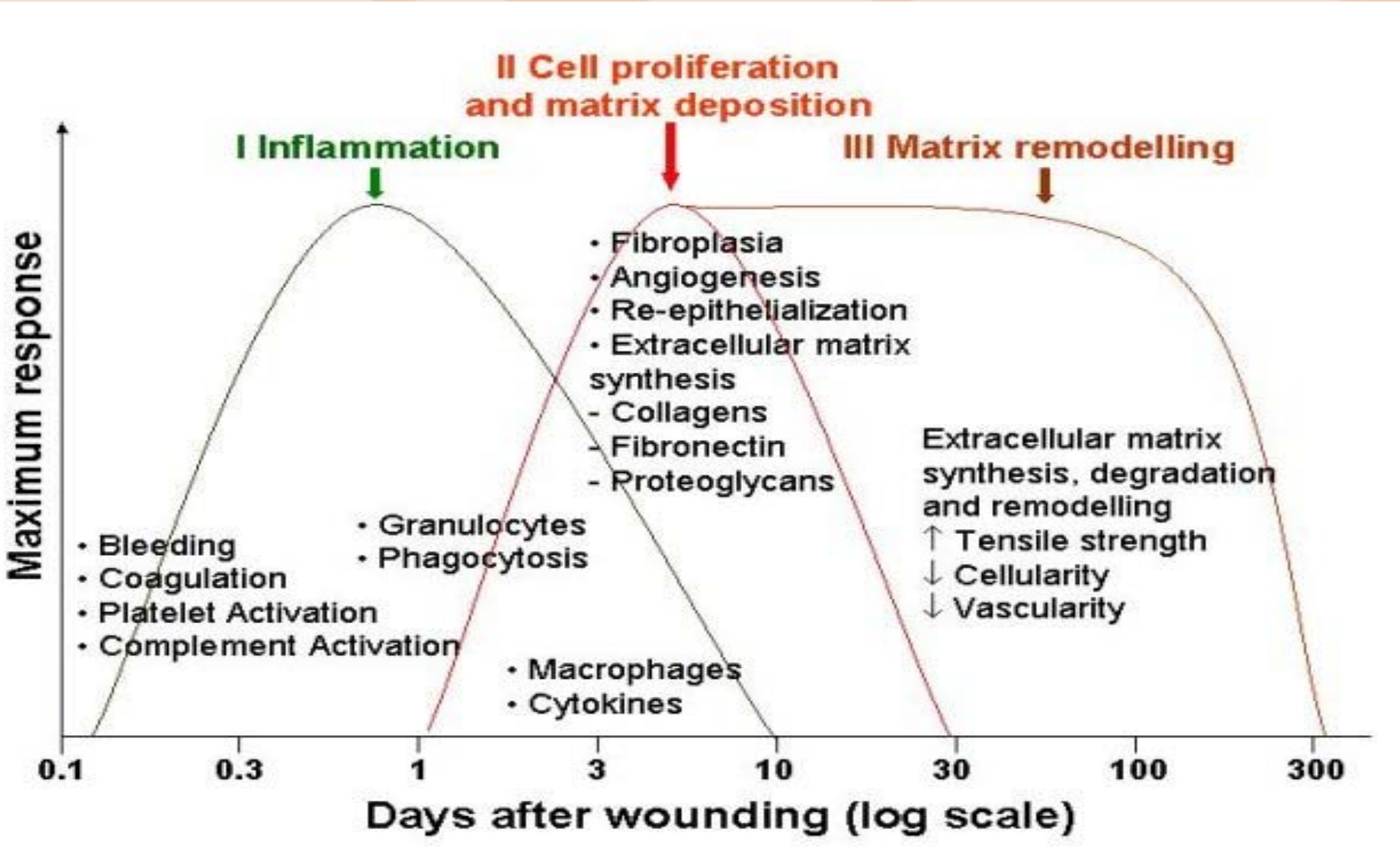
Patient Selection: Timelines in Wound Healing



Patient Selection (1)

Patient Selection:

From approximately age 27 years, our bodies produce less collagen and elastin and especially the skin of the face looses elasticity and begins to ‘sag’. This process is complicated by our environment (UV light exposure), our lifestyle (excess alcohol and smoking), medication, disease & genetic pre-disposition

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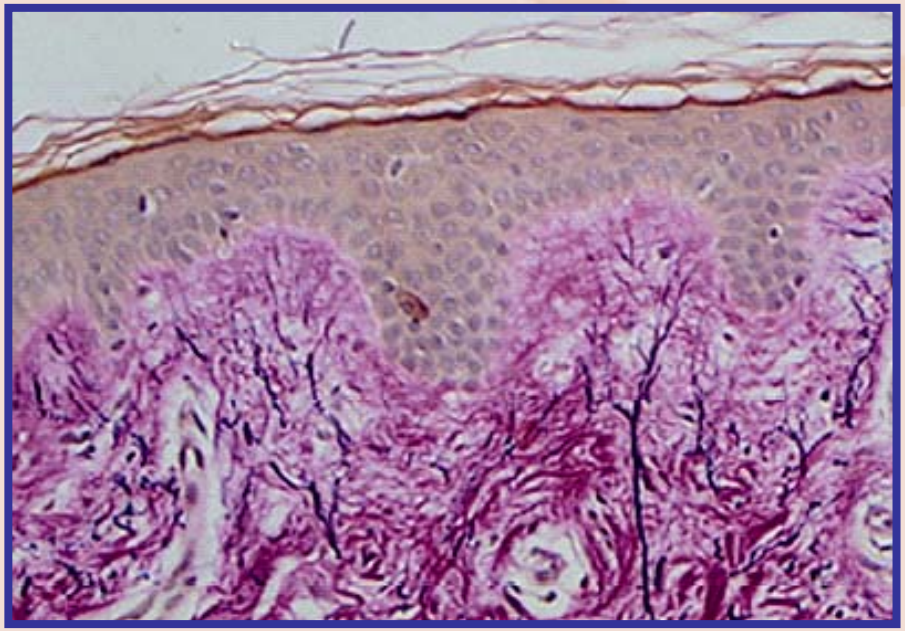
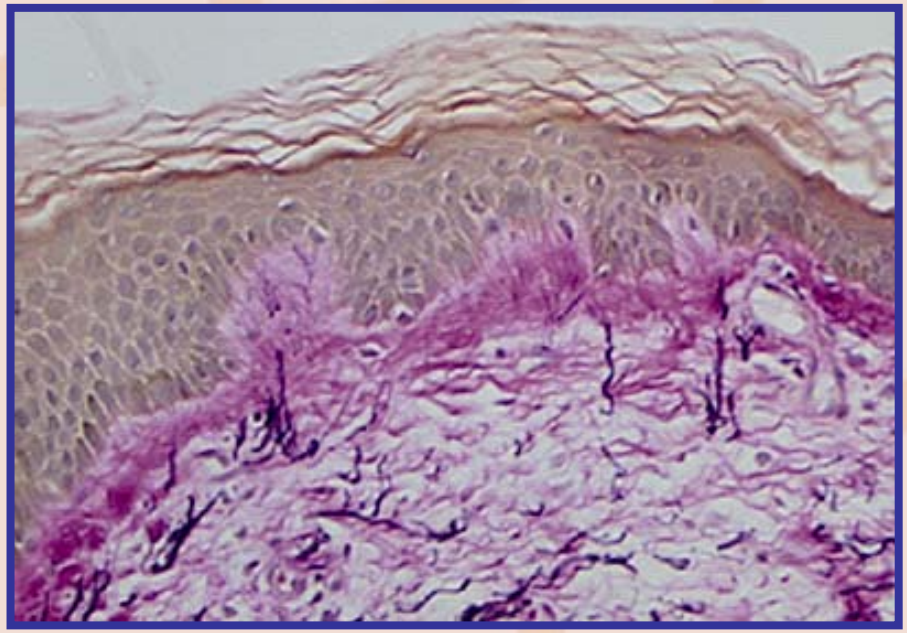
What happens when we get older ?

24 years old



48 years old



Elastica von Giesson staining

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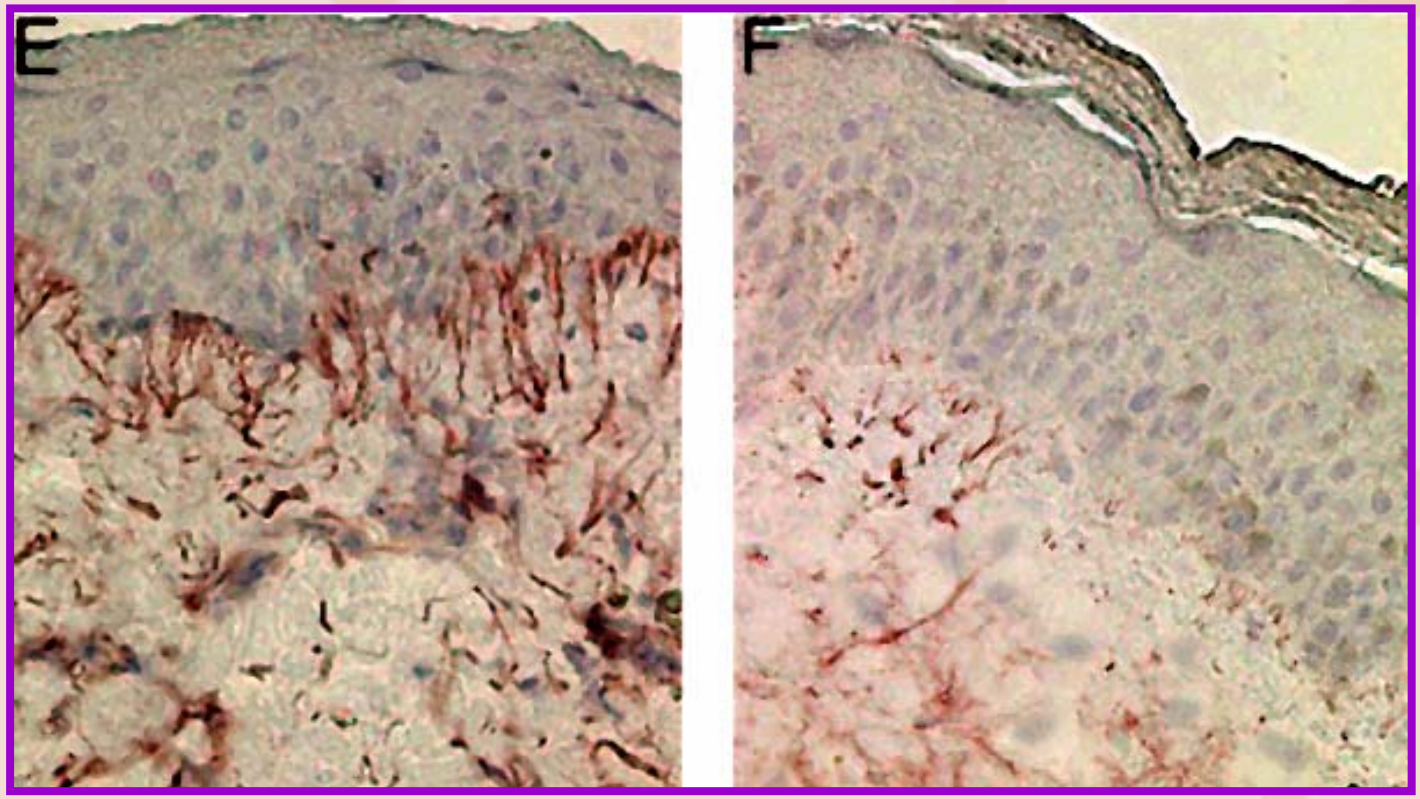
Elastolysis: degeneration of elastic fibers

26 years old

66 years old



Specific immuno-elastin staining

Patient Selection (2)

Age 30 – 40 years: In this age category the main aim is rejuvenation and prevention of collagen degradation and re-stimulation of new collagen and elastin production, thus attempting to slow down the ageing process. One treatment every 2 years is sufficient.

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Patrient Selection (3)

- **Age 40 – 50 years:** In this age category the aim is rejuvenation and to attempt reversal of the ageing process which is more difficult. More re-stimulation of collagen and elastin production is necessary. The initial treatment should be followed by a second treatment 3-6 months later and a third treatment 9 months after the second treatment. Follow-up ‘maintenance’ treatments may be necessary every 12-18 months.

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Patient Selection (4)

Age 50+ years: In this age category the aim is rejuvenation and to attempt more intense reversal of the ageing process. The initial treatment should be followed by a second treatment 3-6 months later and a third treatment 6 months after the second treatment. Follow-up 'maintenance' treatments may be necessary every 12 months.

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