MyCells® - Platelet Rich Plasma harvesting kit : from the benchtop to the clinic

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MyCells® LTD, Tel Aviv

What happens when we get older ?

- Damages in Dermis and Epidermis layers
- Decrease in:
  - Content of Elastin & Collagen
  - Skin fatty layers
  - Number of cells
  - Extra cellular matrix

<u>All theses lead to:</u>

- Changes in skin texture, firmness, radiance, volume, flexibility and wrinkle formation

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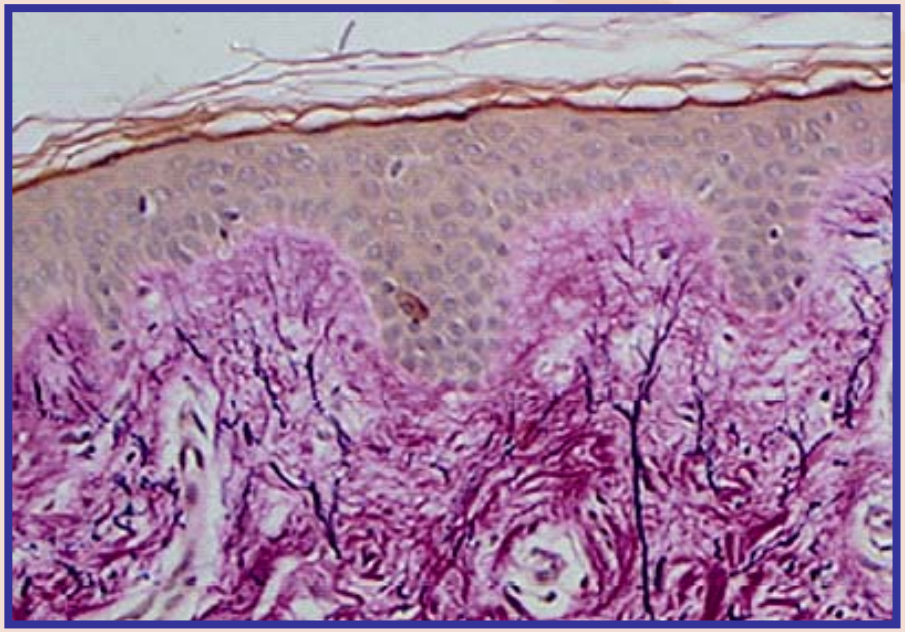
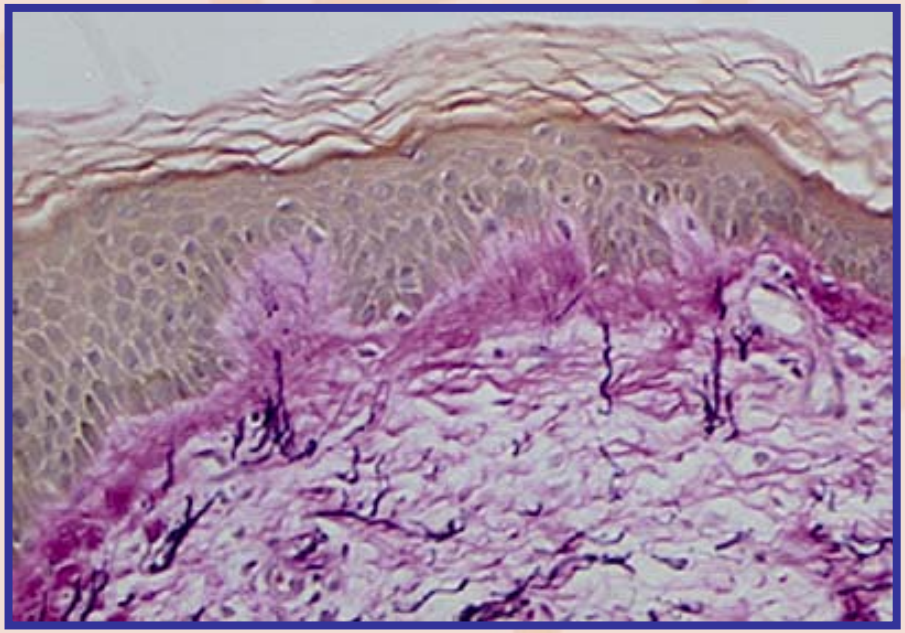
What happens when we get older ?

24 years old



48 years old



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Current Solutions for Skin Rejuvenetion:

DERMO COSMETICIS

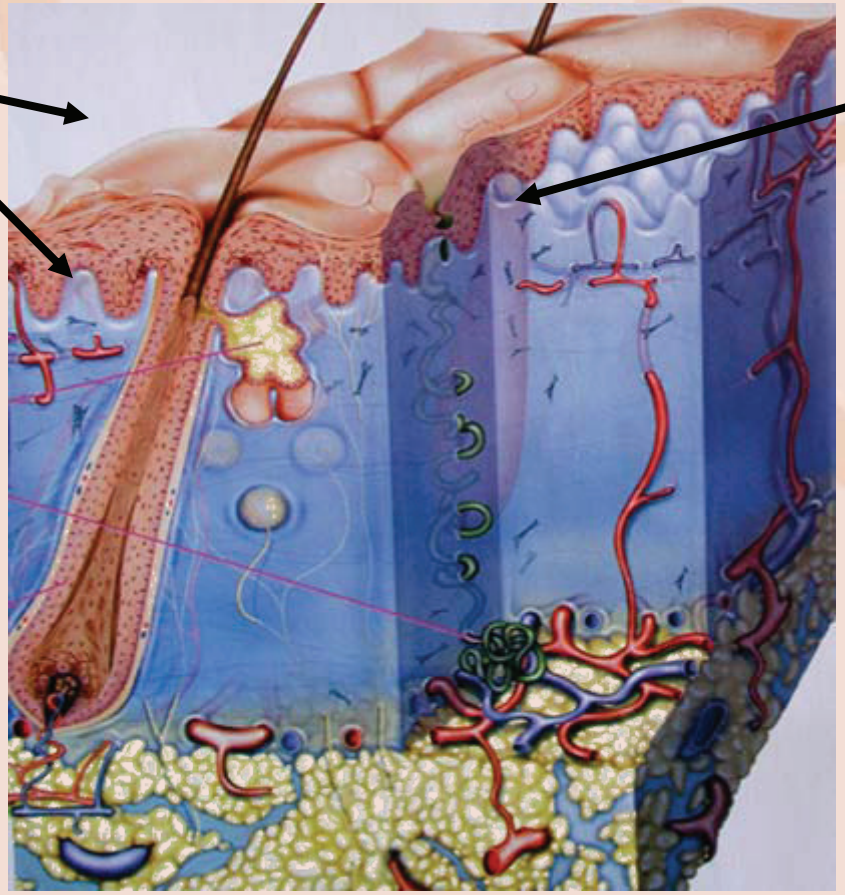
PEELS

Renewal of the corneal layer

Germinative layer Stimulation

Dermis Stimulation

Hyperpigmentation removal



FILLERS

Wrinkles & volumes correction

Plastic Surgery

MyCells®

Current market Fillers are:

- Foreign to the body:
  - Animals/ Synthetic (Collagen)
  - Synthetic (HA)
  - Bacteria (Botox)
- Limited life span (reabsorbed by the body)
- Limited reconstitution function of the damaged tissue.

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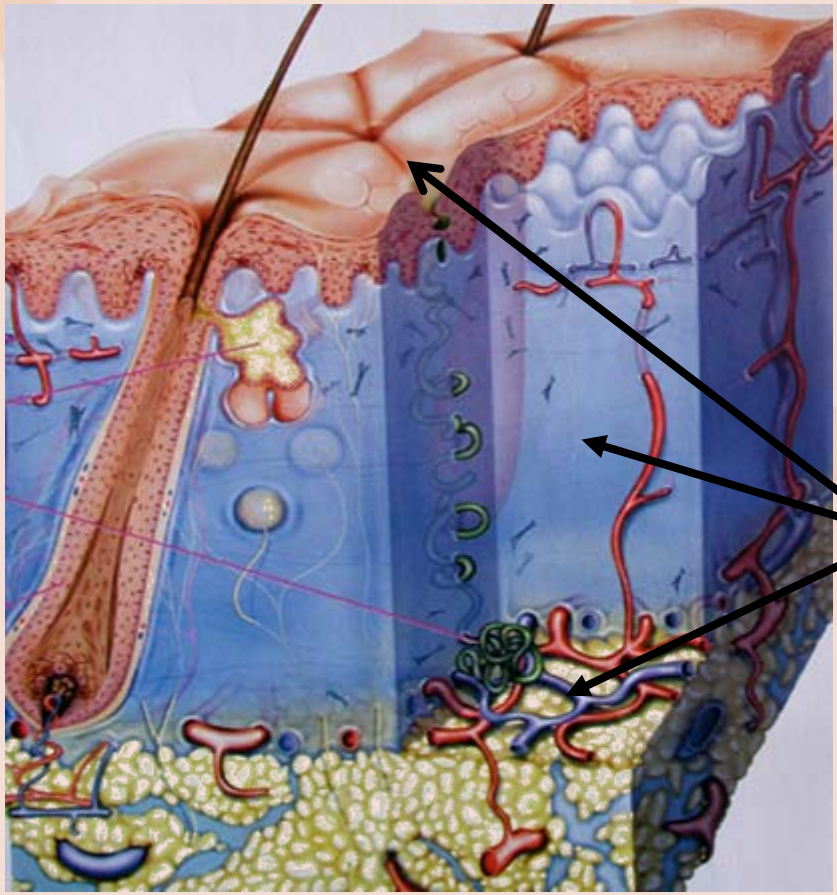
Presenting MyCells® :

Bio stimulation with Platelet Rich plasma (PRP)



MyCells® : **BIO STIMULATION**

Architectural skin reconstruction/ reorganization

MyCells®

MyCells®– a revolutionary method allowing rejuvenation of the skin, using the patient's own (autologous) cells, with long lasting effect.

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MyCells® PRP harvesting kit - Visible Results:

Transformation of Mr. Suzuki with 6 PRP injections in 18 months

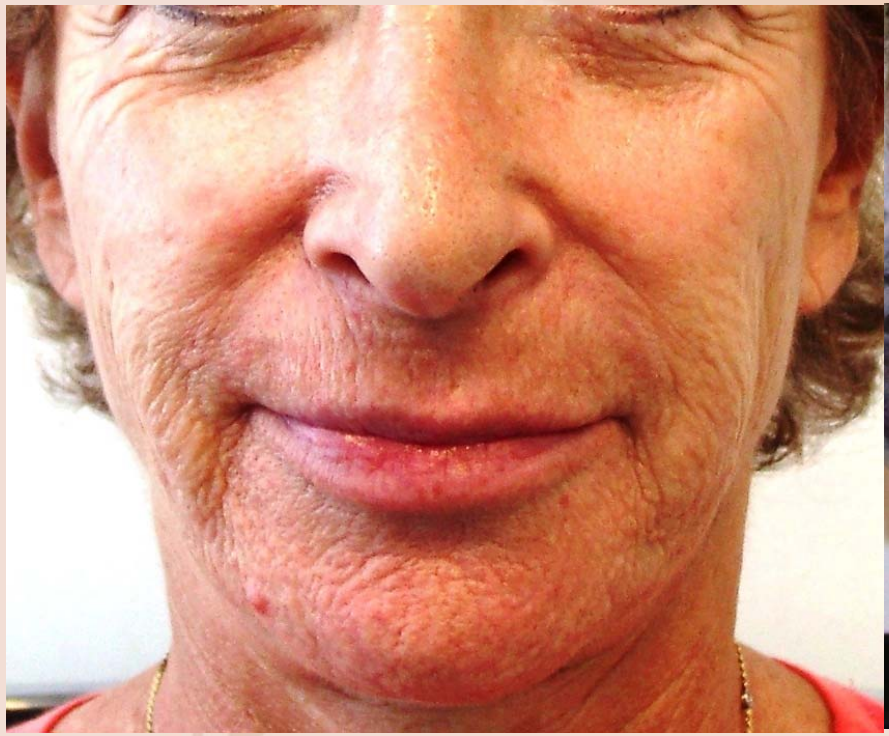
Dec. 2006

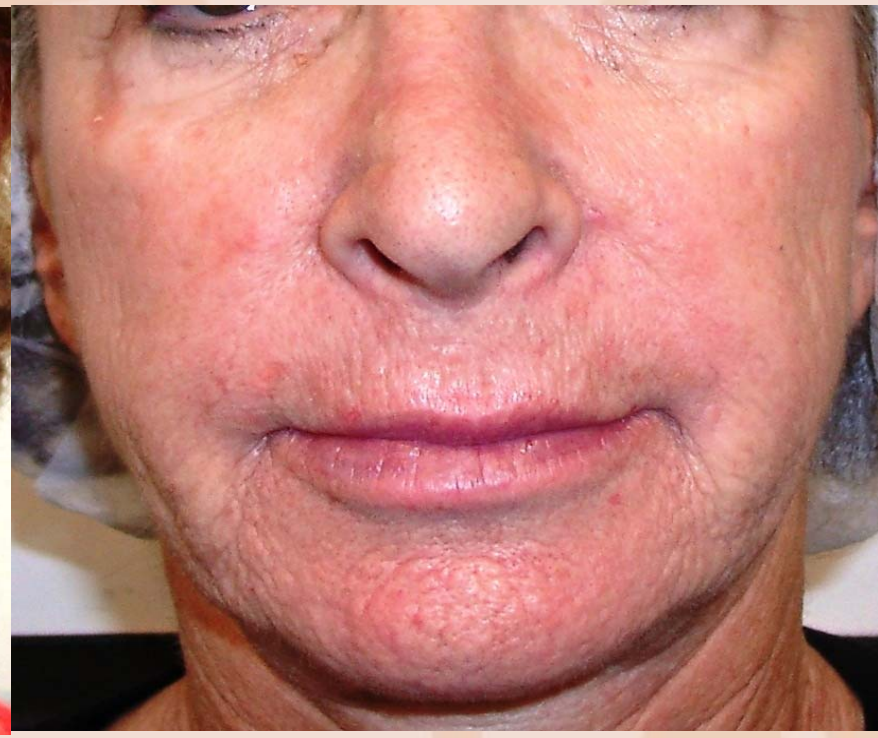
March 2007

June 2008

MyCells® PRP harvesting kit - Visible Results:

Aged 60: 21 months after 1 injx.





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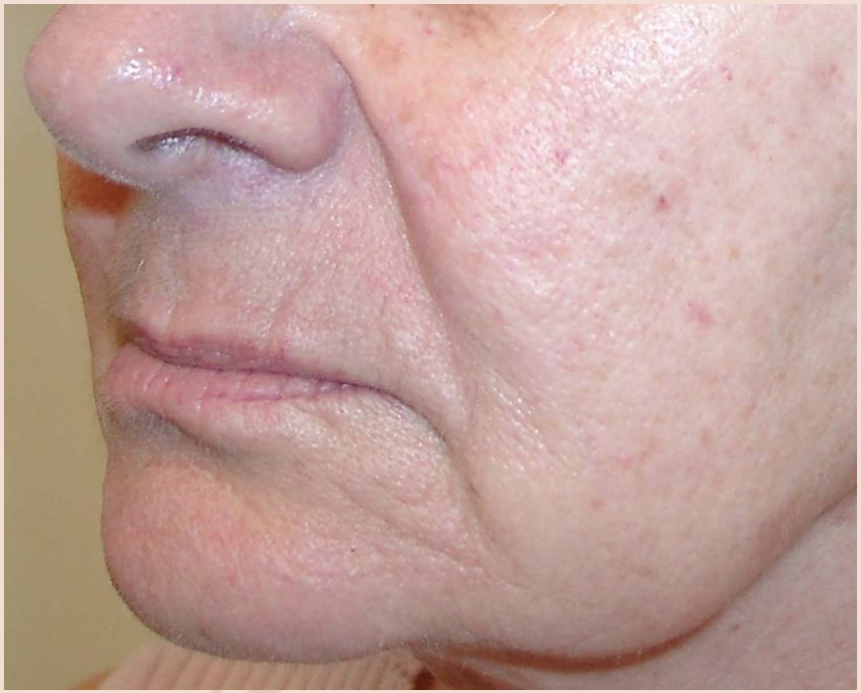
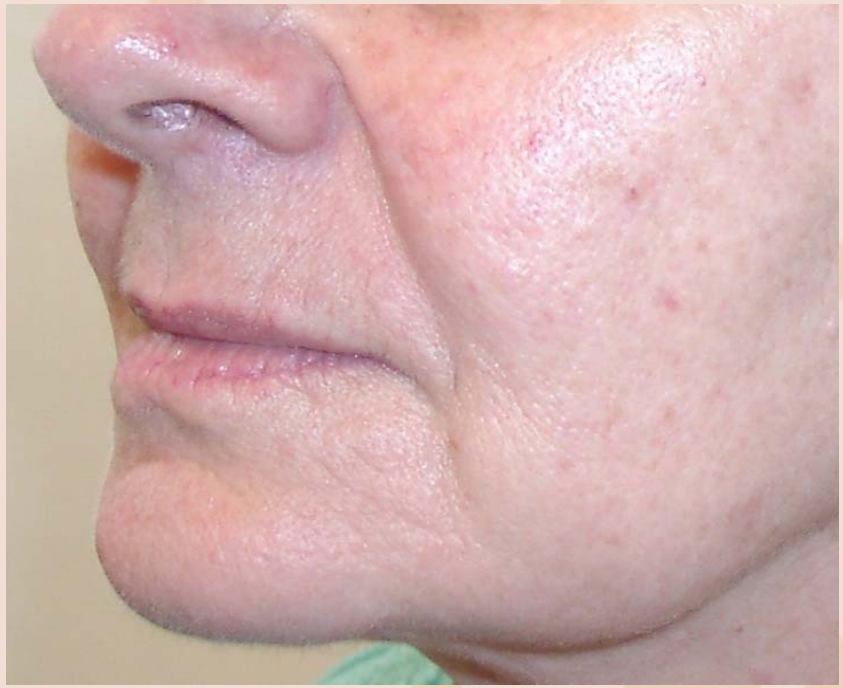
MyCells® PRP harvesting kit - Visible Results:

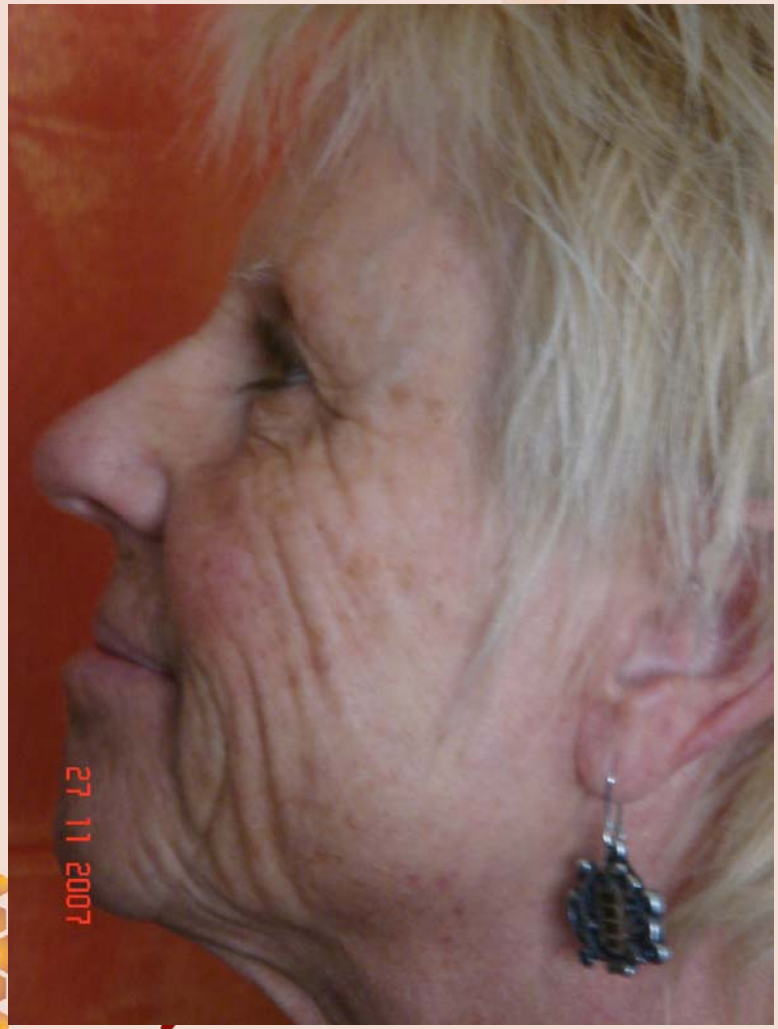
Aged 69: 2 months after 1 injx.

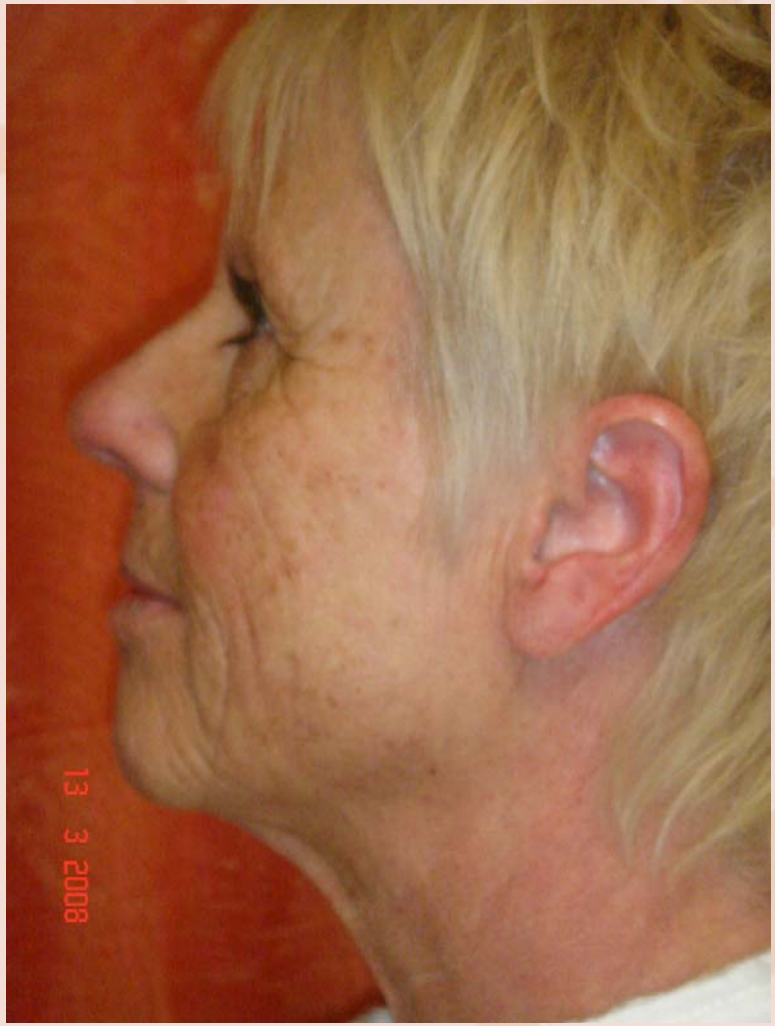




MyCells® PRP harvesting kit - Visible Results:

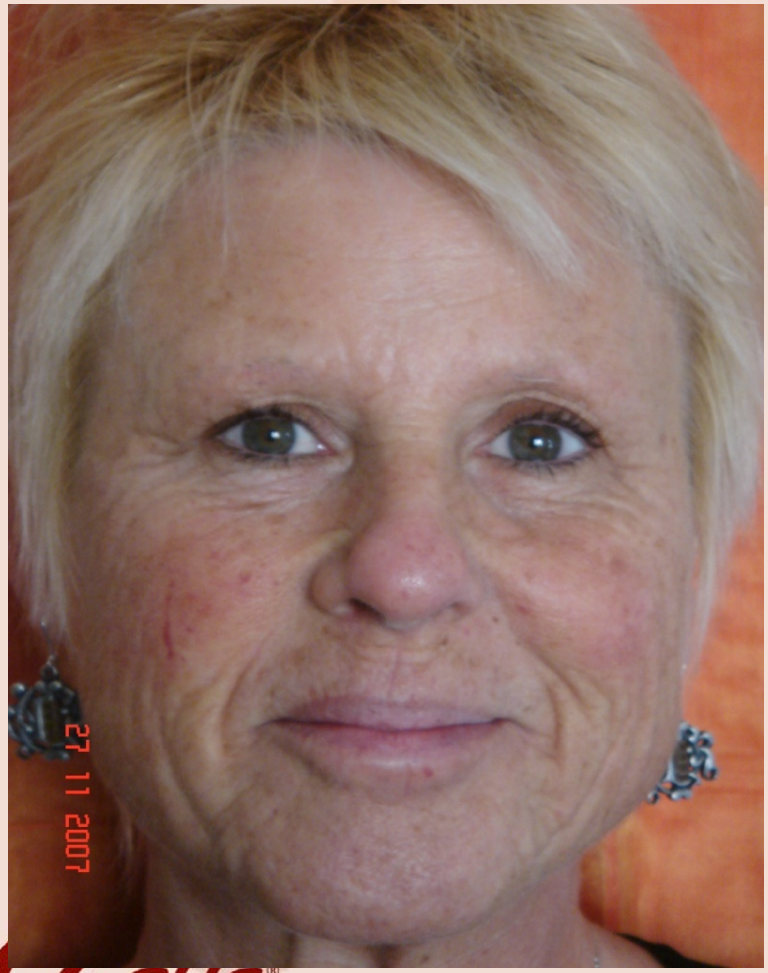
3 months after 1 injx

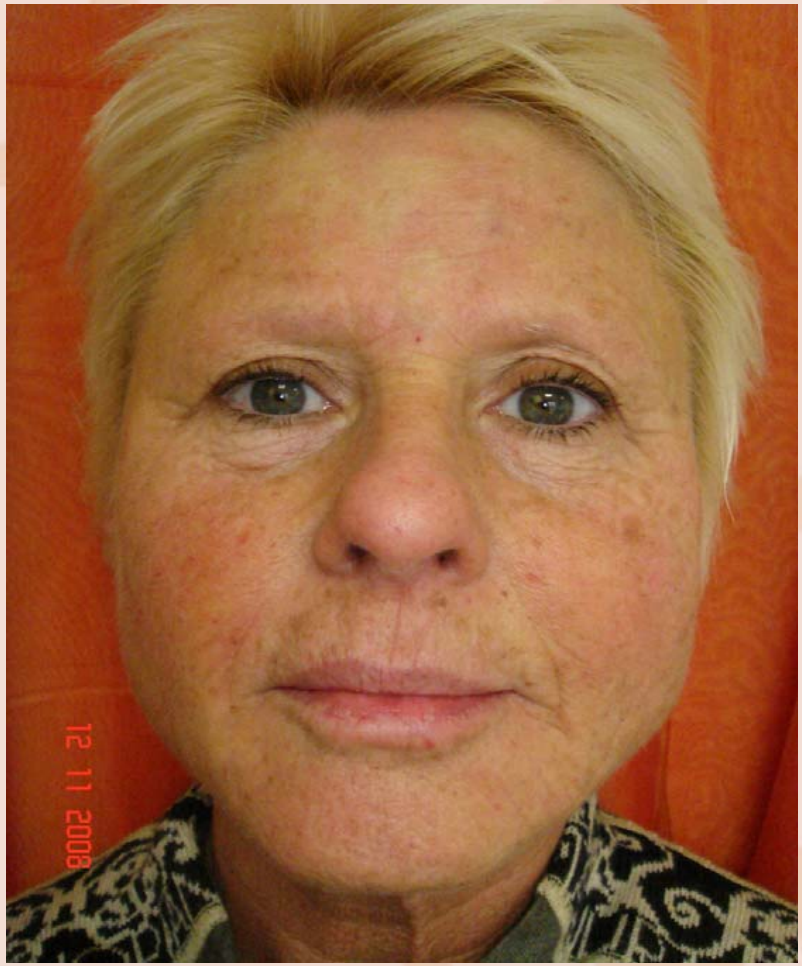




MyCells® PRP harvesting kit - Visible Results:

1 Year after 1 injx.





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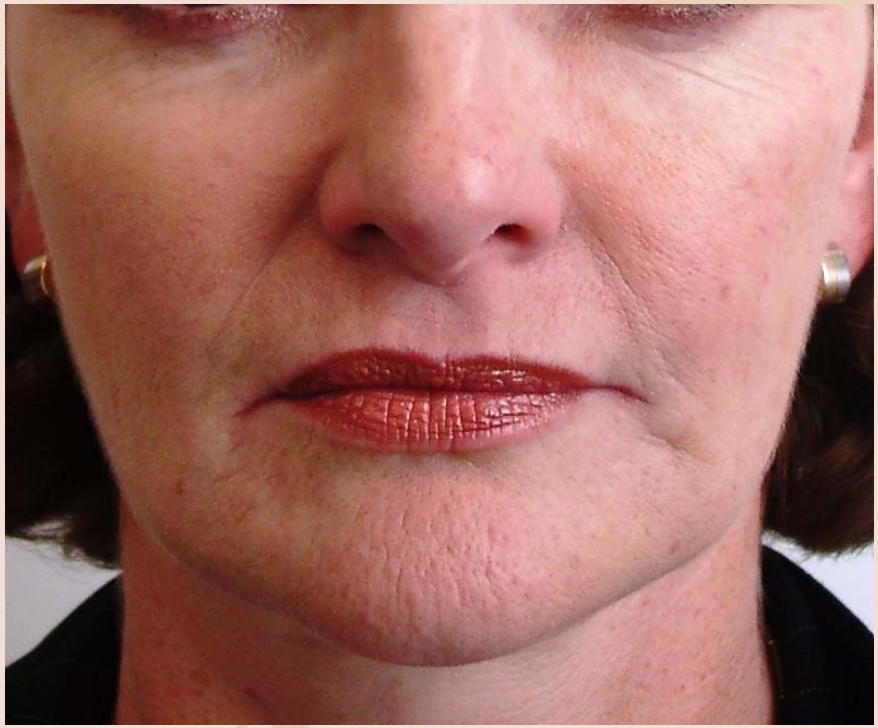
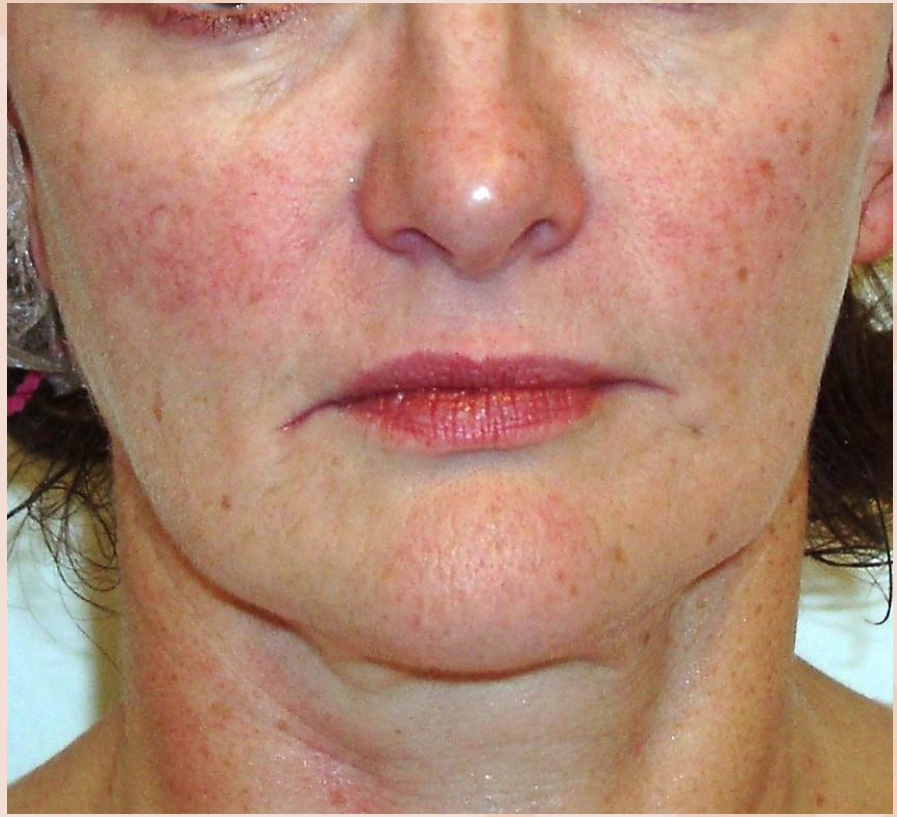
MyCells® PRP harvesting kit - Visible Results:

54 years: 2 months after 1 injx.





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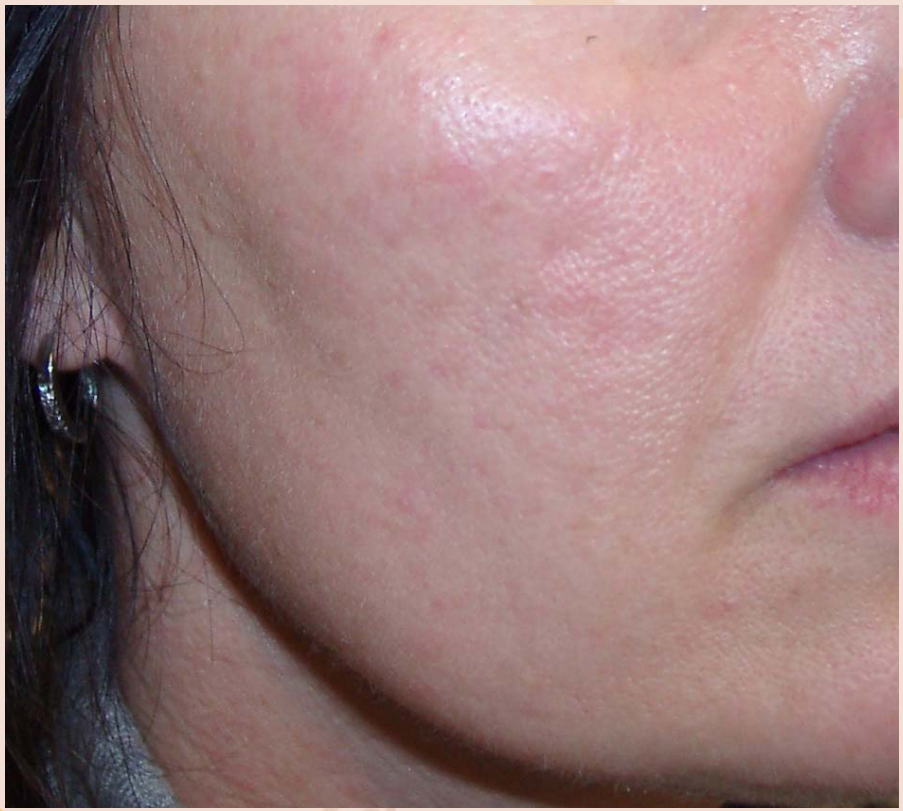
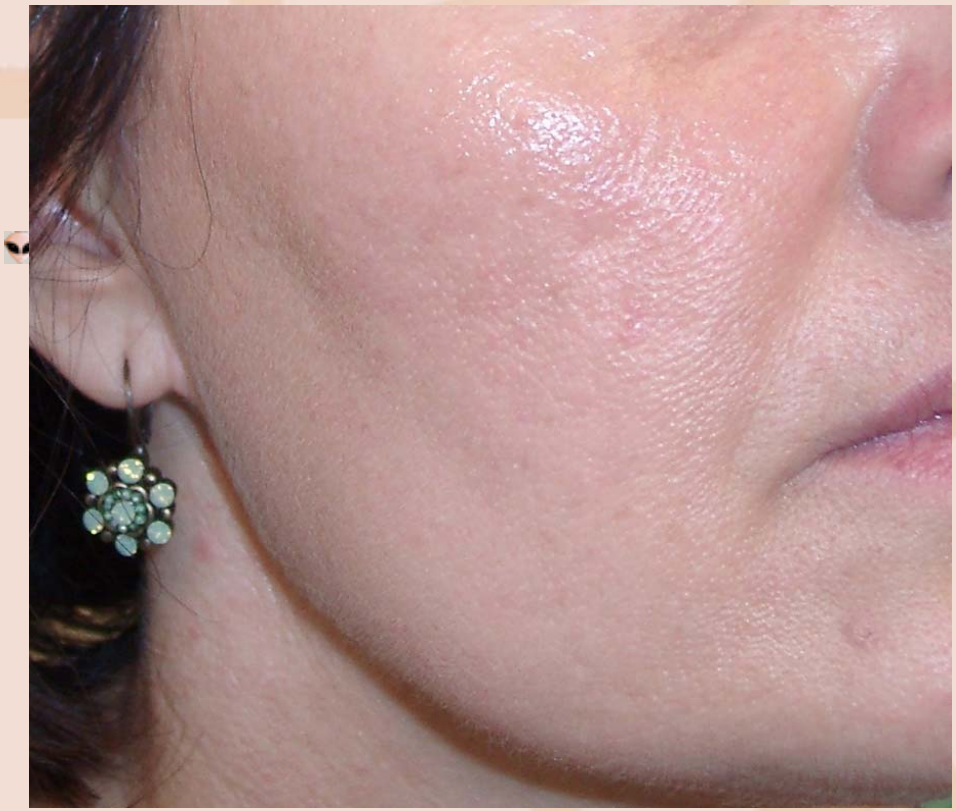
MyCells® PRP harvesting kit - Visible Results:

46 years: Acne scars - 3 months after 2 injx.





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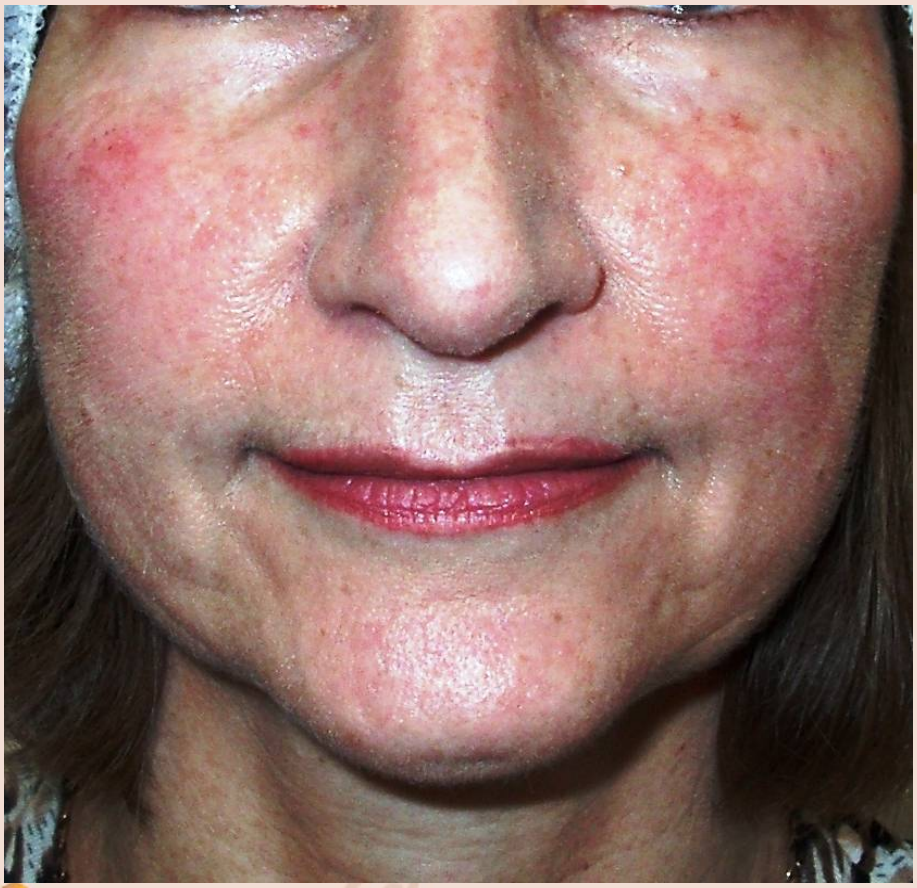
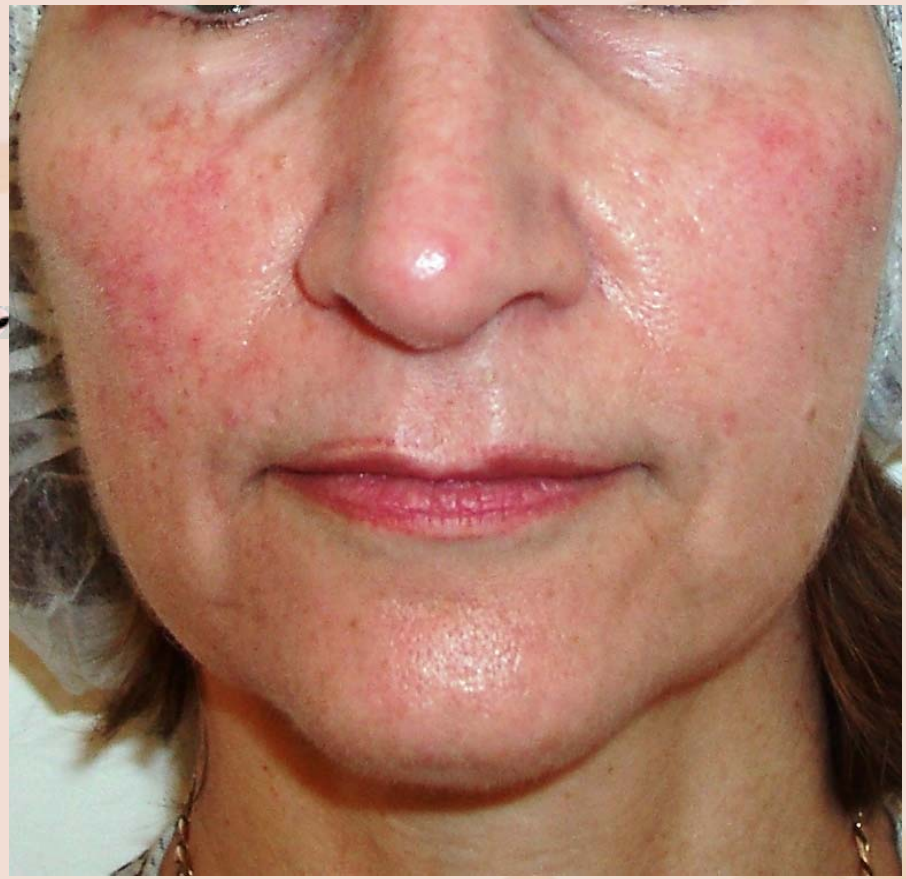
MyCells® PRP harvesting kit - Visible Results:

51 years: 2 months after 1 injx

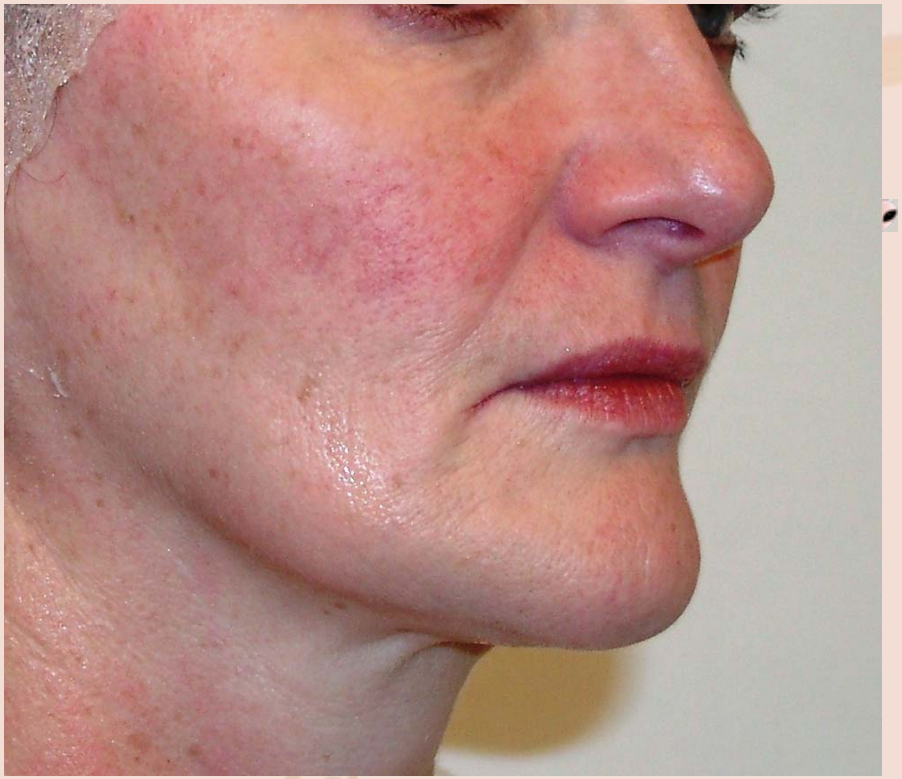


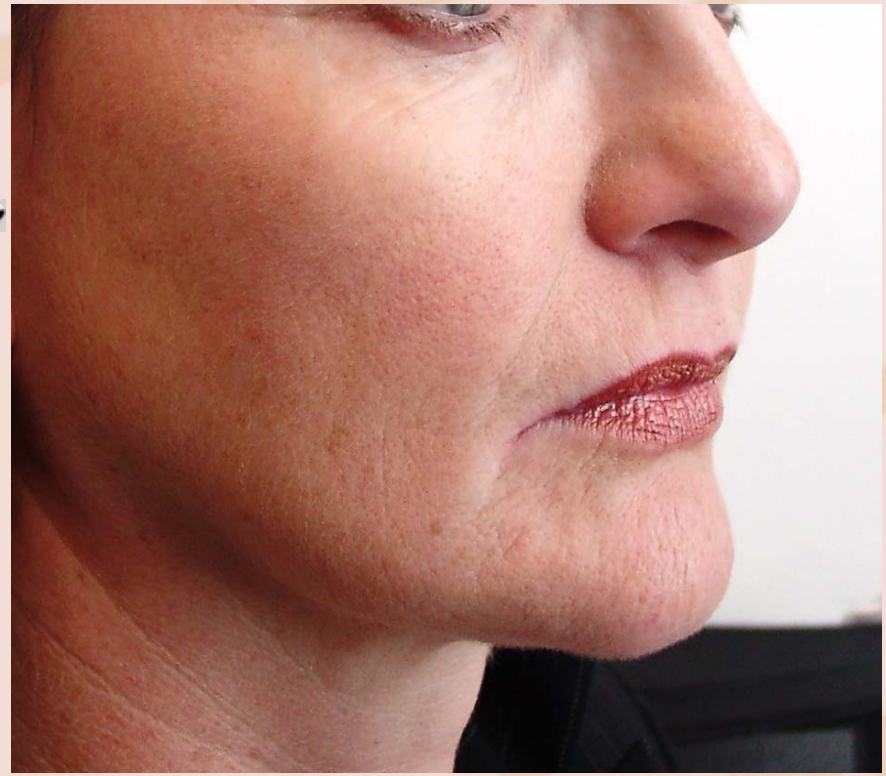


MyCells®

MyCells® PRP harvesting kit - Visible Results:

54 years: 2 months after 2 injx.





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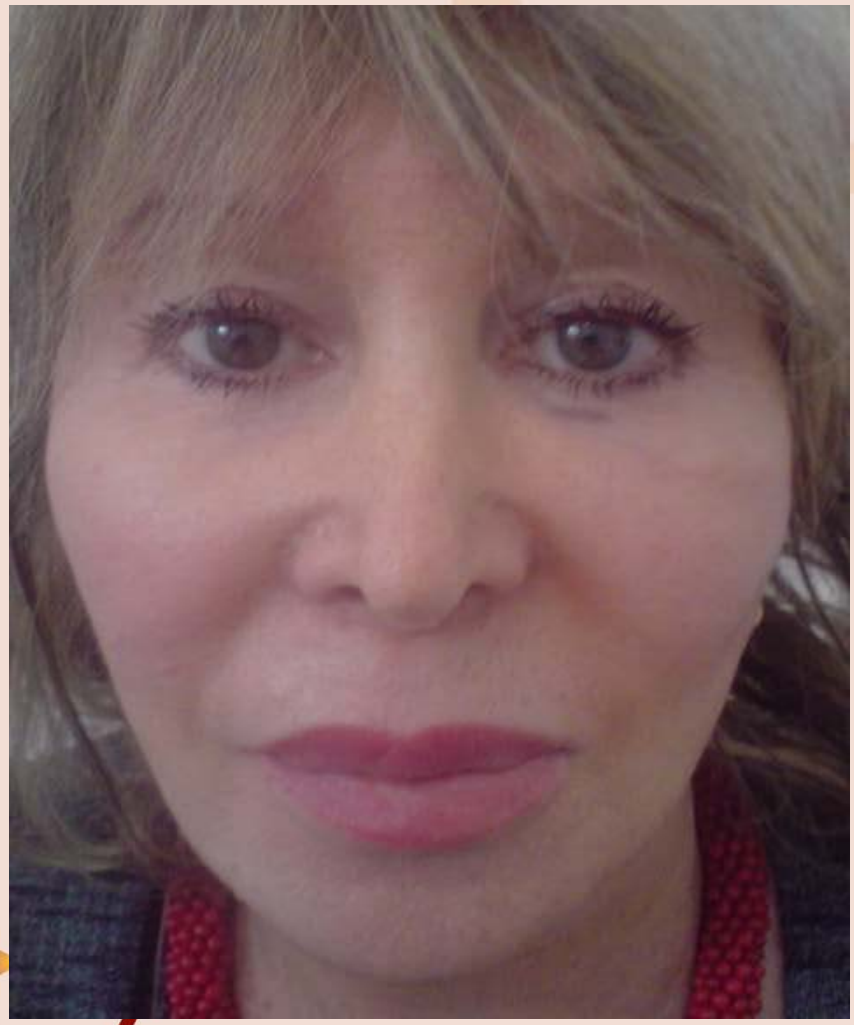
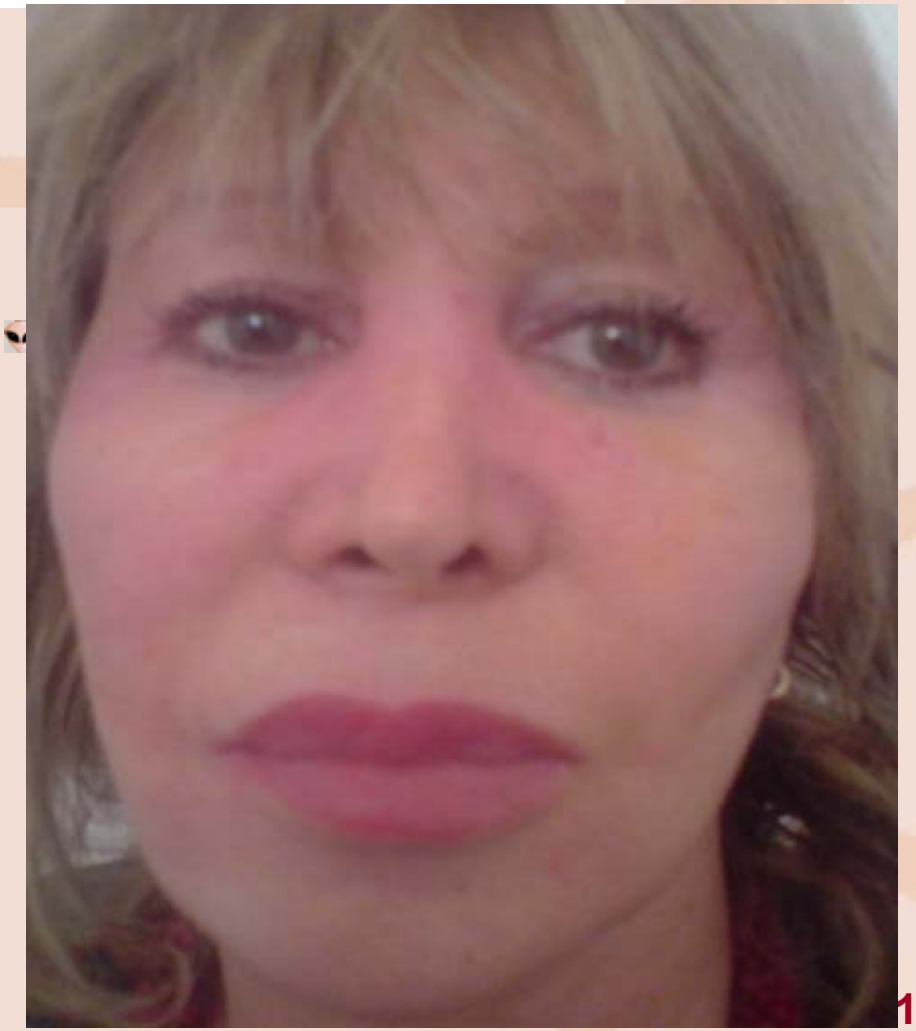
MyCells® PRP harvesting kit - Visible Results:

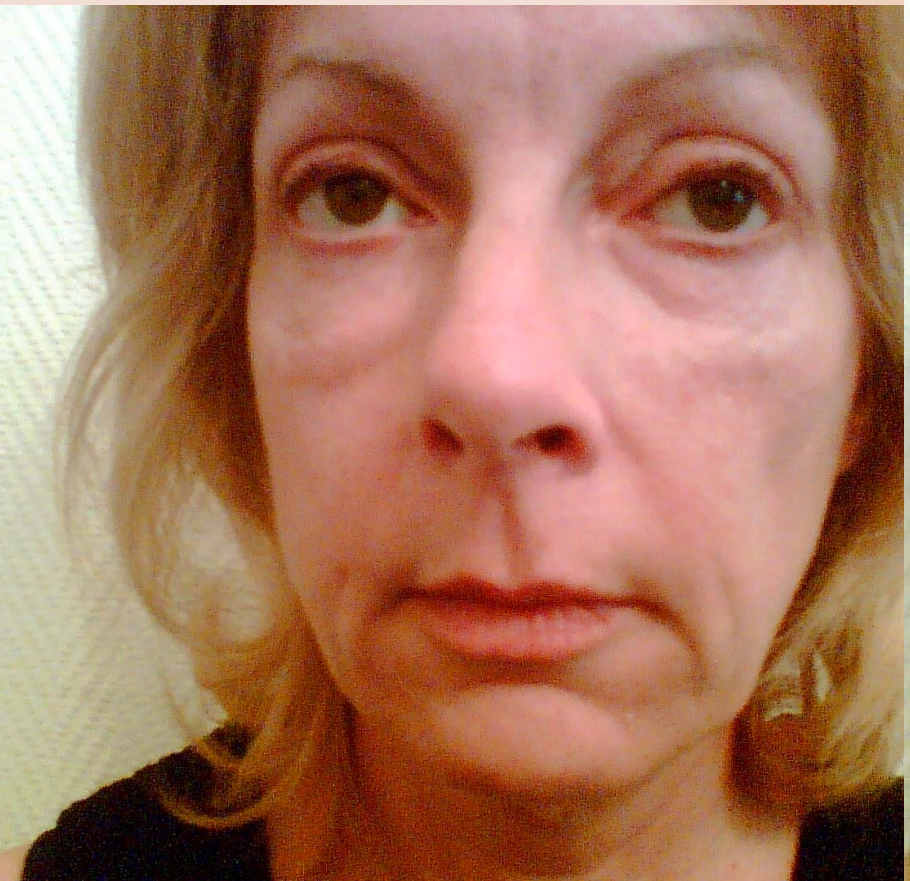
- **50 years: 4 months after 1 injx.**

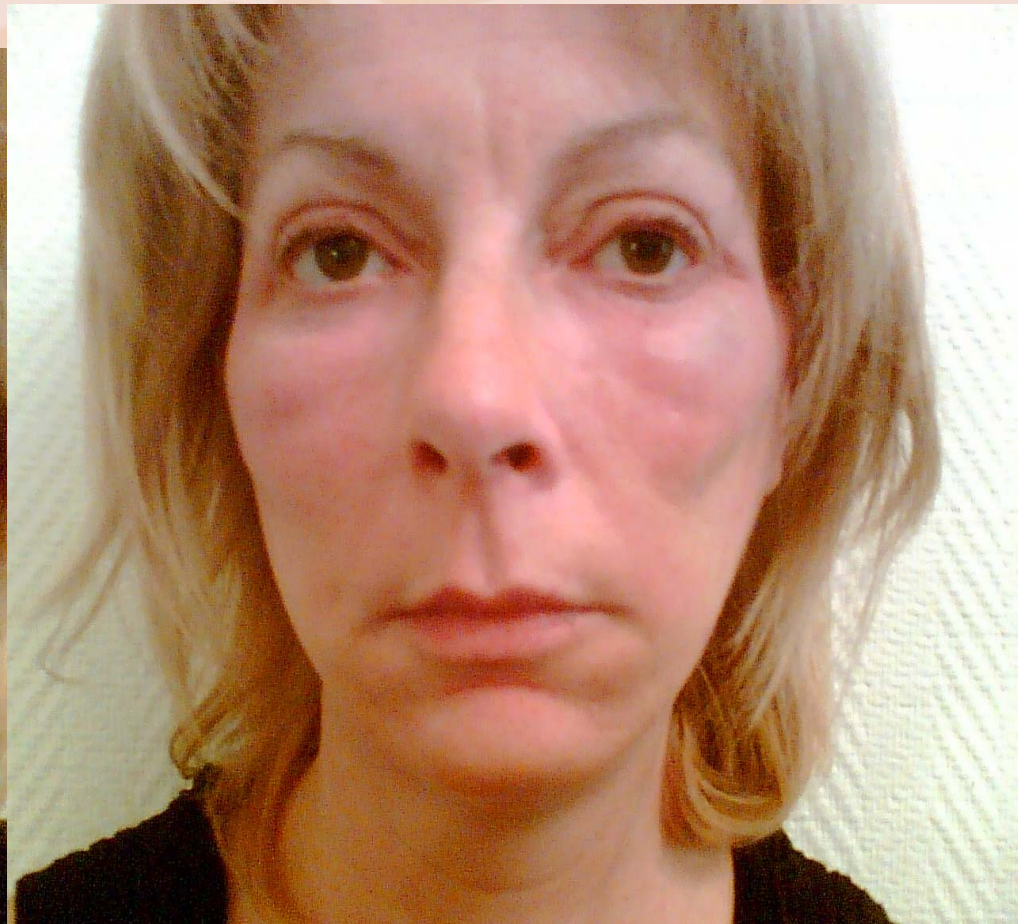




MyCells® PRP harvesting kit - Visible Results:

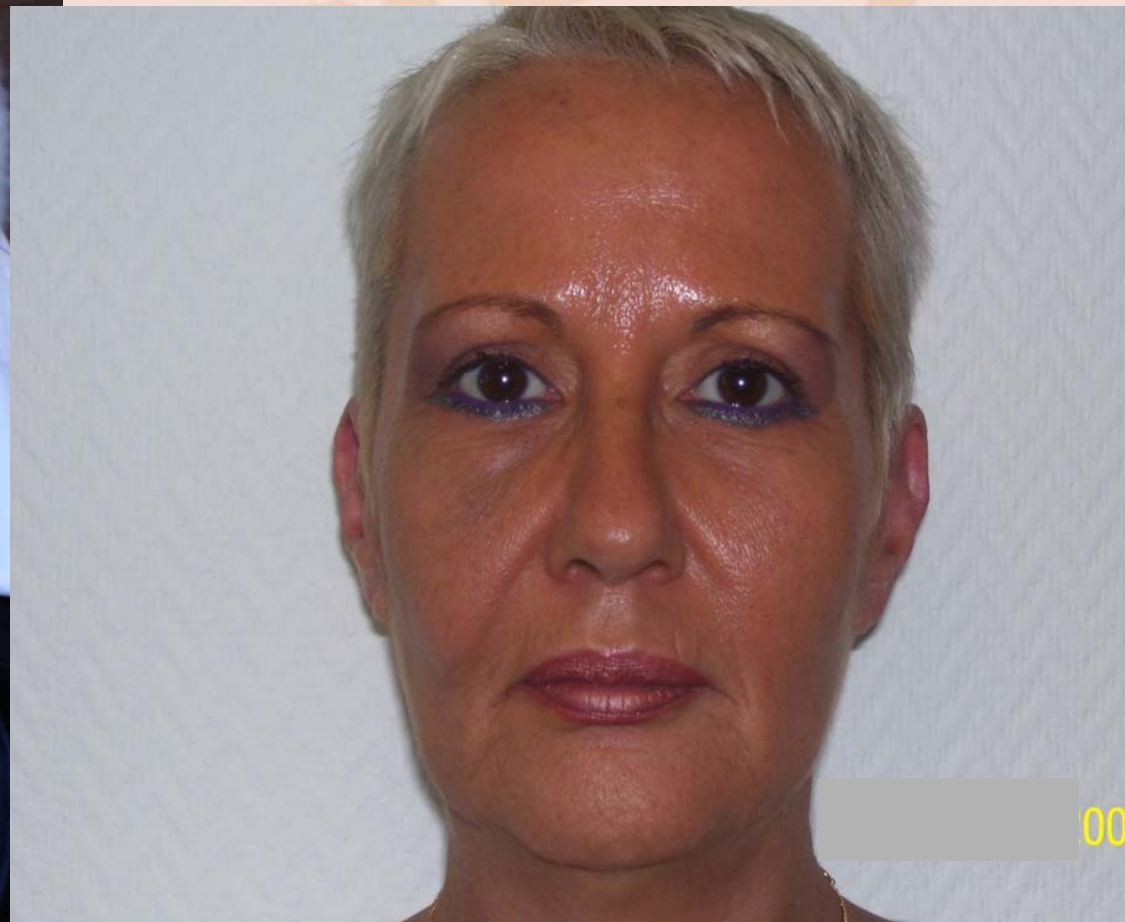
55 years: 4 months after 1 injx.





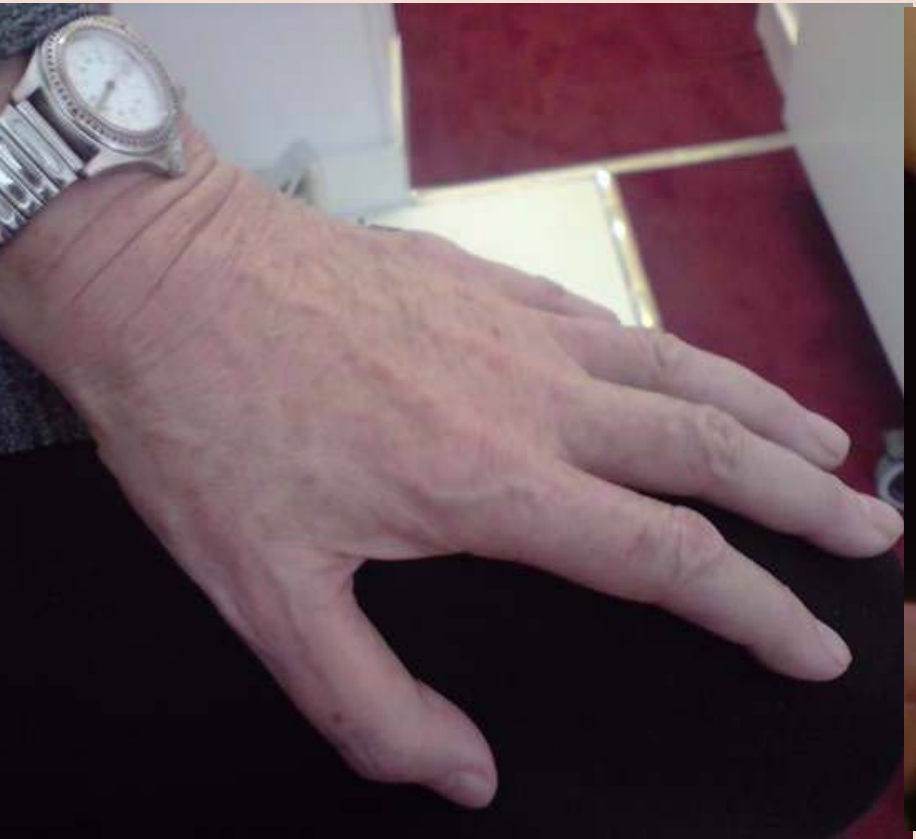
MyCells® PRP harvesting kit - Visible Results:

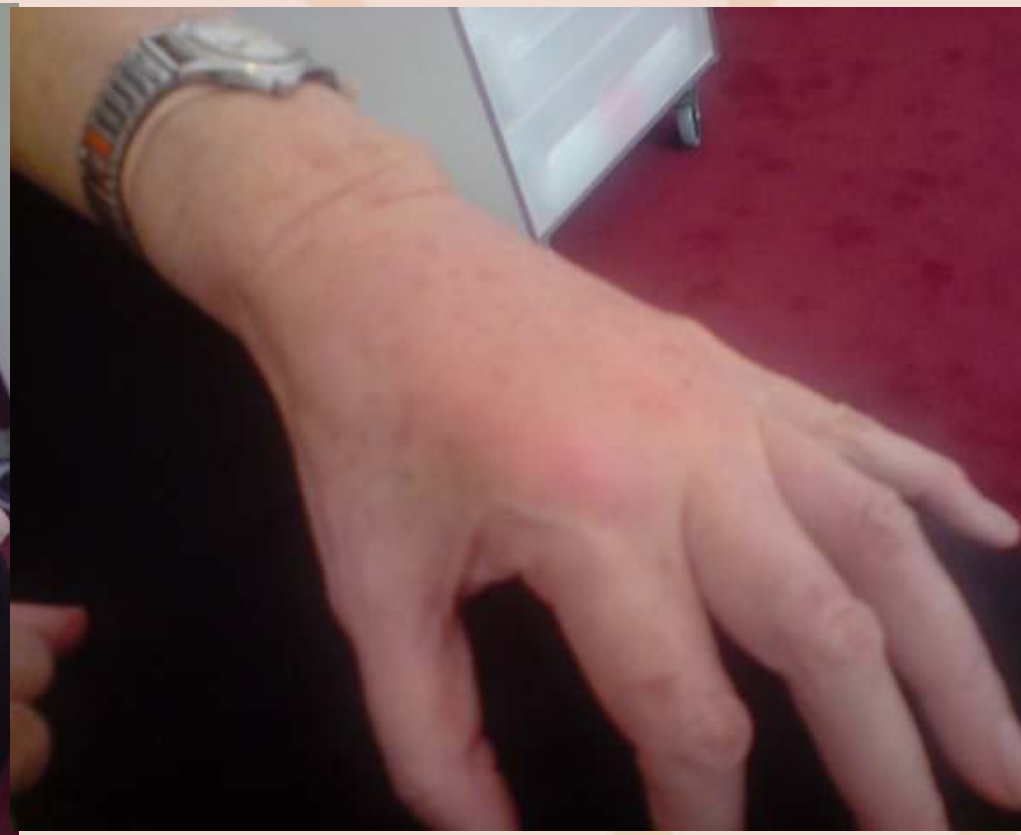
54 years: 3 months after 2 injx.



MyCells® PRP harvesting kit - Visible Results:

2 months after 2 injx.





What is PRP?

- PRP (Platelet Rich plasma) –

  a concentration of human platelets (PLT) in a small volume of plasma (x 2-3 concentrated).

- PRP utilizes the patients own (autologous) PLT, derived from his/her blood.

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Why is the excitement about PRP?

- PRP usage – takes advantage of normal healing pathways – only at an accelerated rate.
- During wound healing, many cells rush into the wound site.
- Among these cells, there are platelets (PLT).

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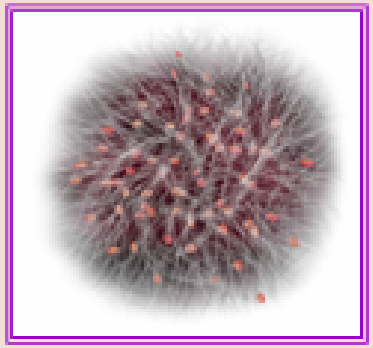
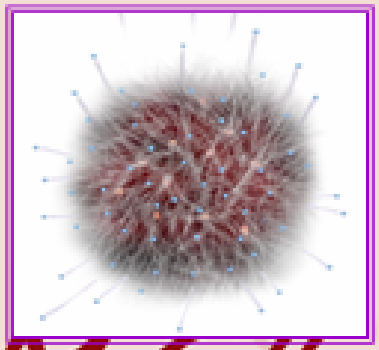
The 5 Major Steps In The Platelet Activation Process

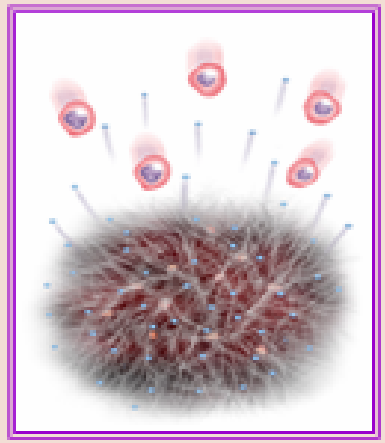
1. Formation of tri-dimensional mesh (fibrin strand) or EXTRA CELLULAR MATRIX



2. De-granulation:
Release of growth factors by the thrombocytes and leukocytes….

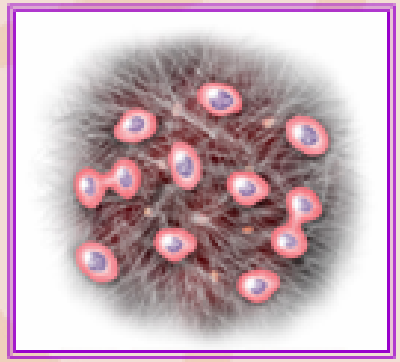


3. Chemo-attraction or migration of macrophages and stem cells…

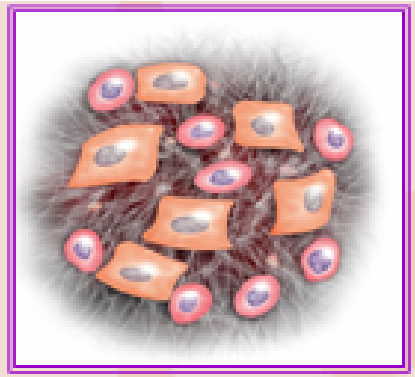


(In addition ECM like fibronection, vitronecton, thrombospondin…)

4. GF bind to Receptors of the cells attracted to the wound - Stem cells proliferation & mitosis…



5. Stem cells differentiation and tissue formations



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

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 ©

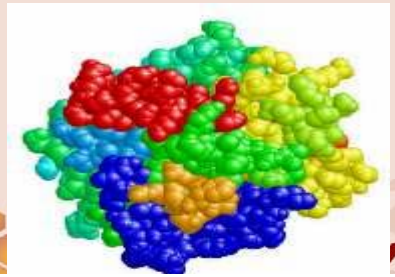
Plasma forms a biological 'scaffold' in-vivo

Via the action of the thrombin…

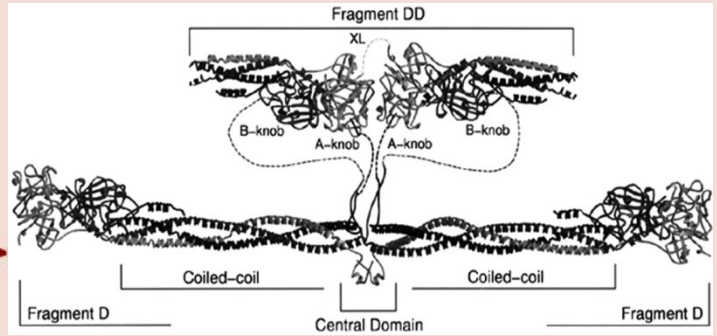
Fibrinogen is transformed to fibrin strands

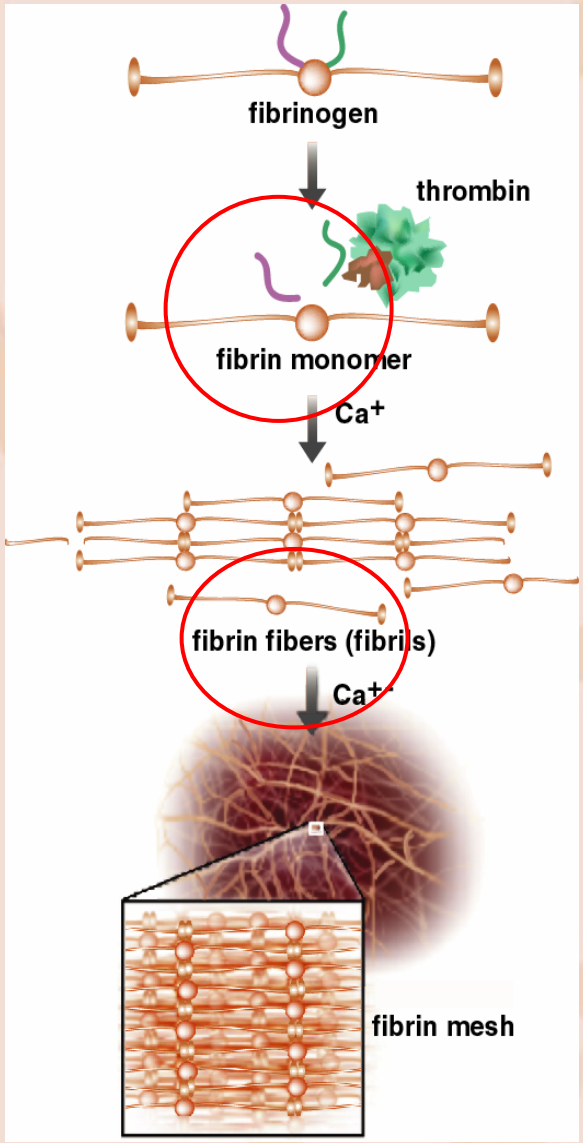
3-D polymeric structure is formed through the binding of fibrin monomers

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



3D Computer Model of Human Thrombin





Fibrinogen: Dr Doolittle

What do PLT do at the wound site?

- **Growth Factors released by PLT:**
  - PDGF:
    - Cells replication
    - Stem cells differentiation
    - Angiogenesis – vascularisation
    - Chemo-attraction of Macrophages, fibroblasts
  - TGFβ:
    - Induction of connective tissue formation
  - ILGF:
    - Wound healing
  - EGF:
    - Induction of cell differentiation

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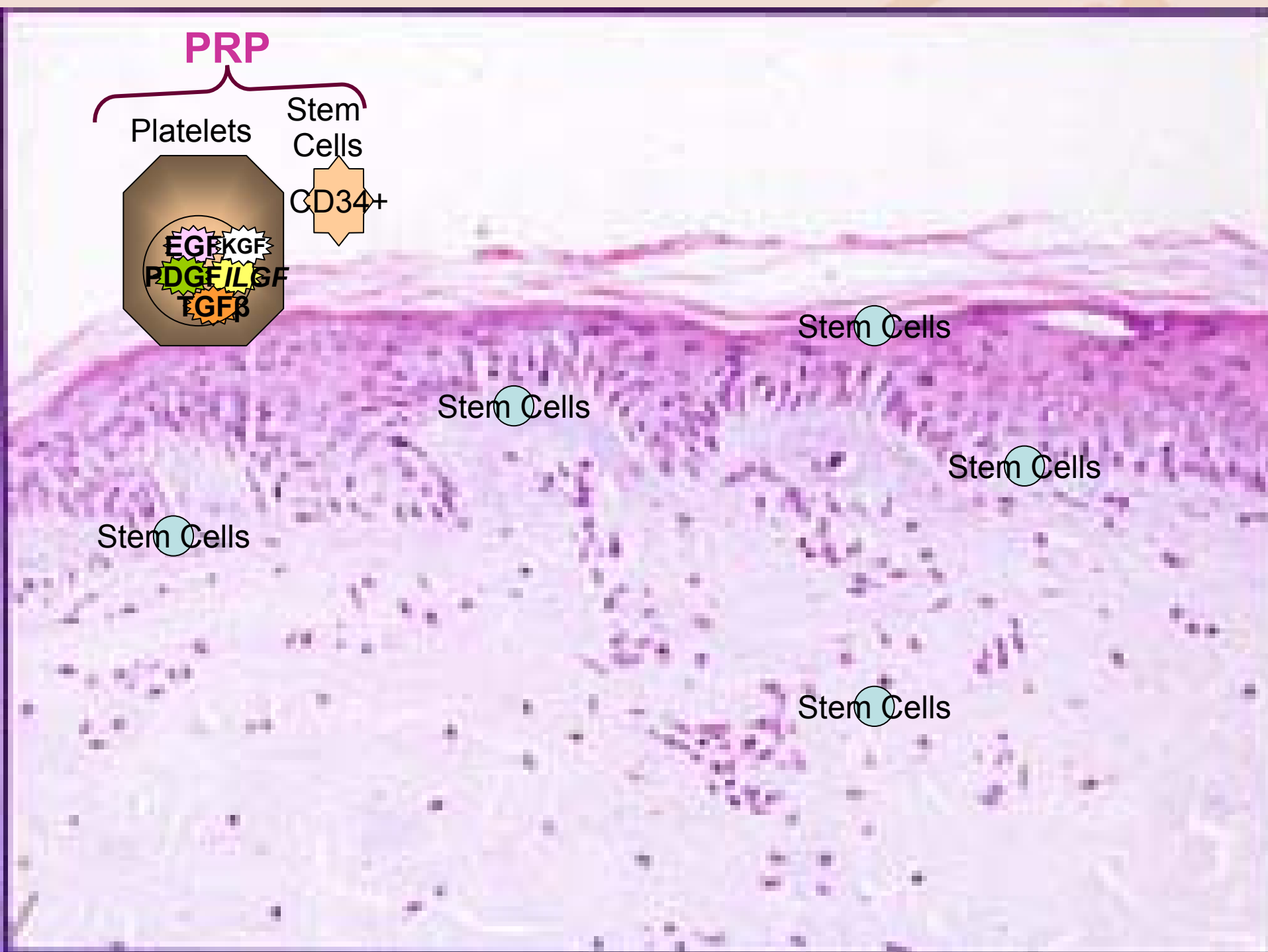
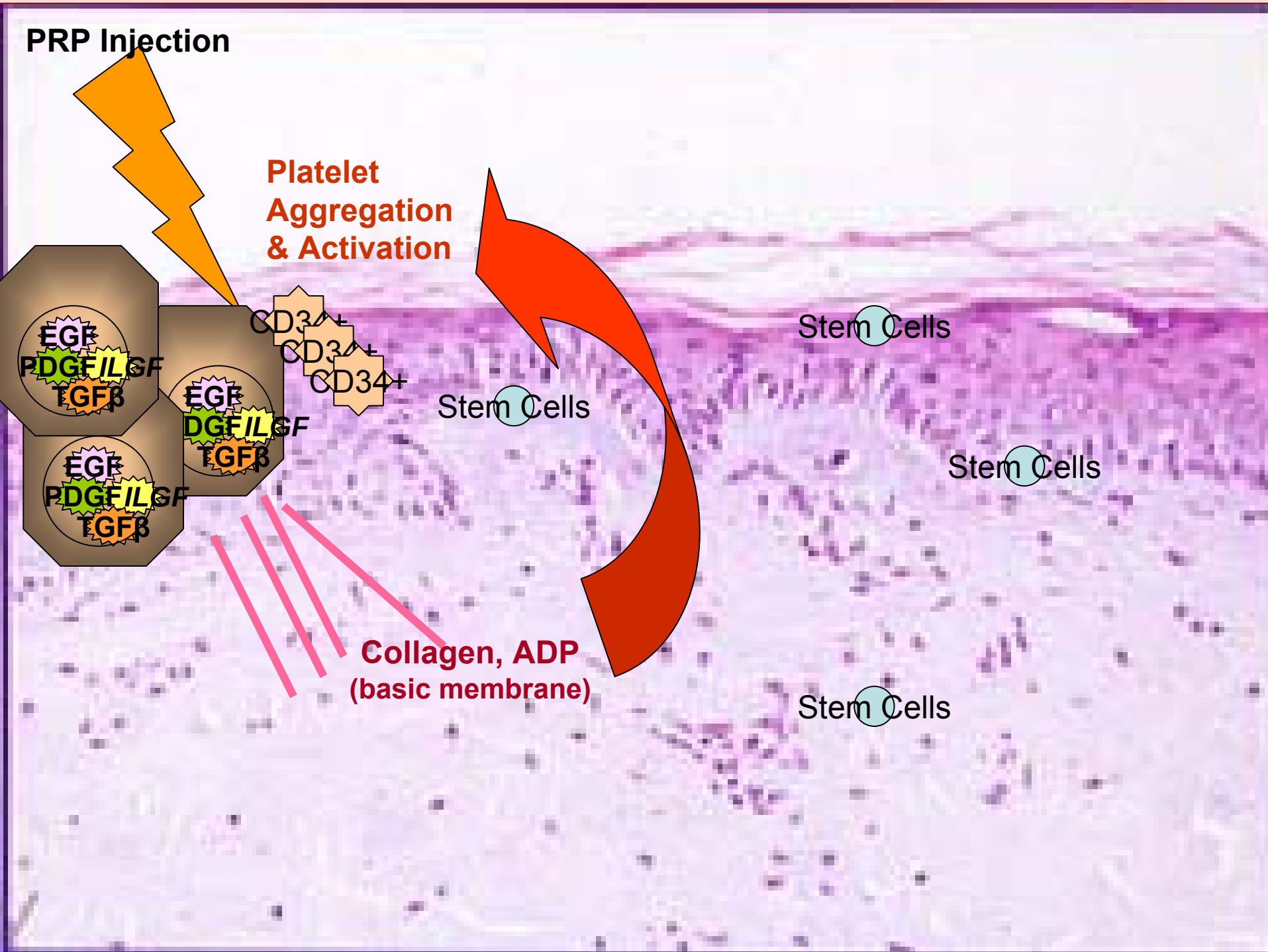
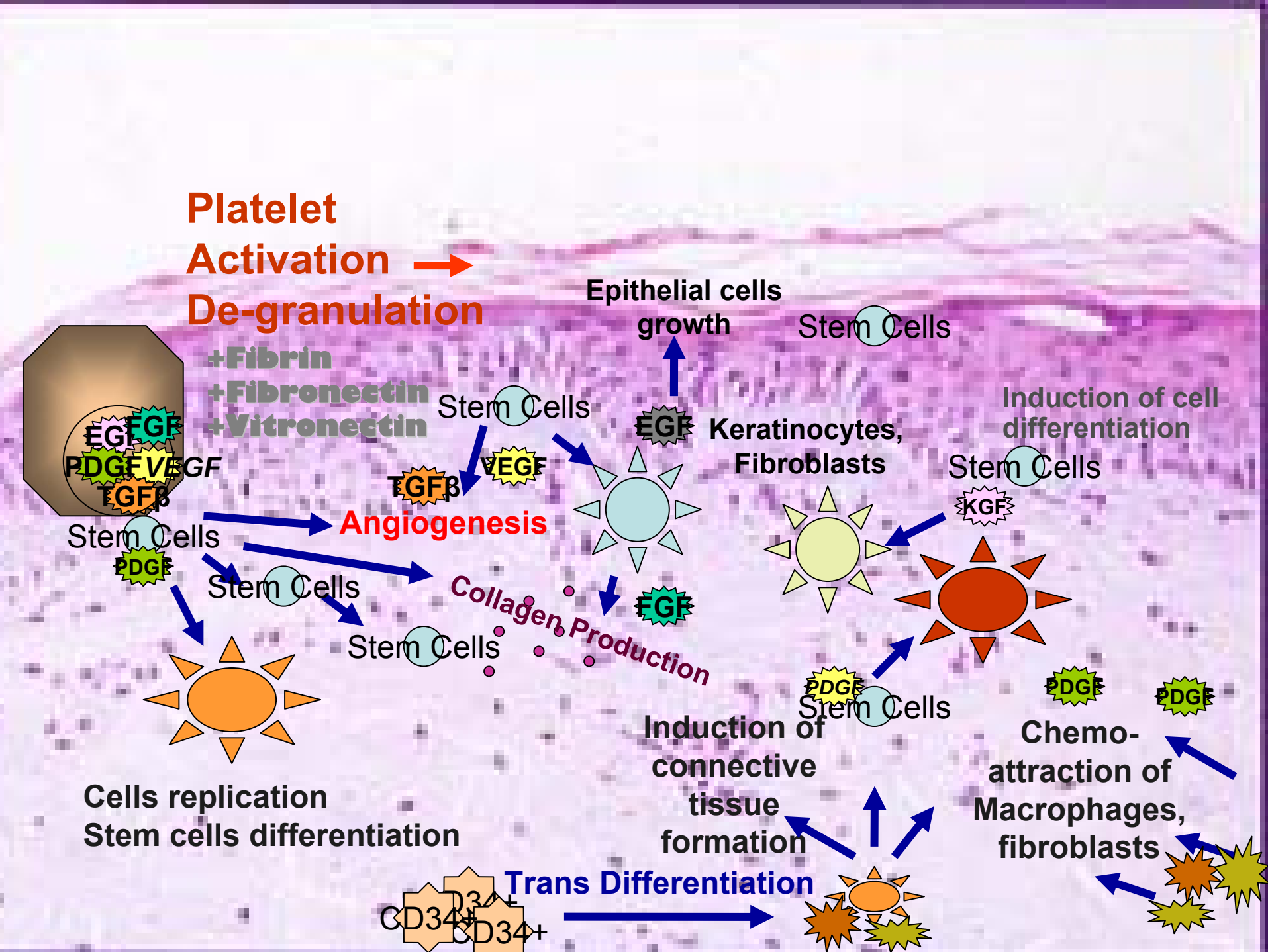
What do PLT do at the wound site?

- PLT also release Proteins that are known to regenerate new tissue:
  - Fibrin
  - Fibronectin
  - Vitronectin

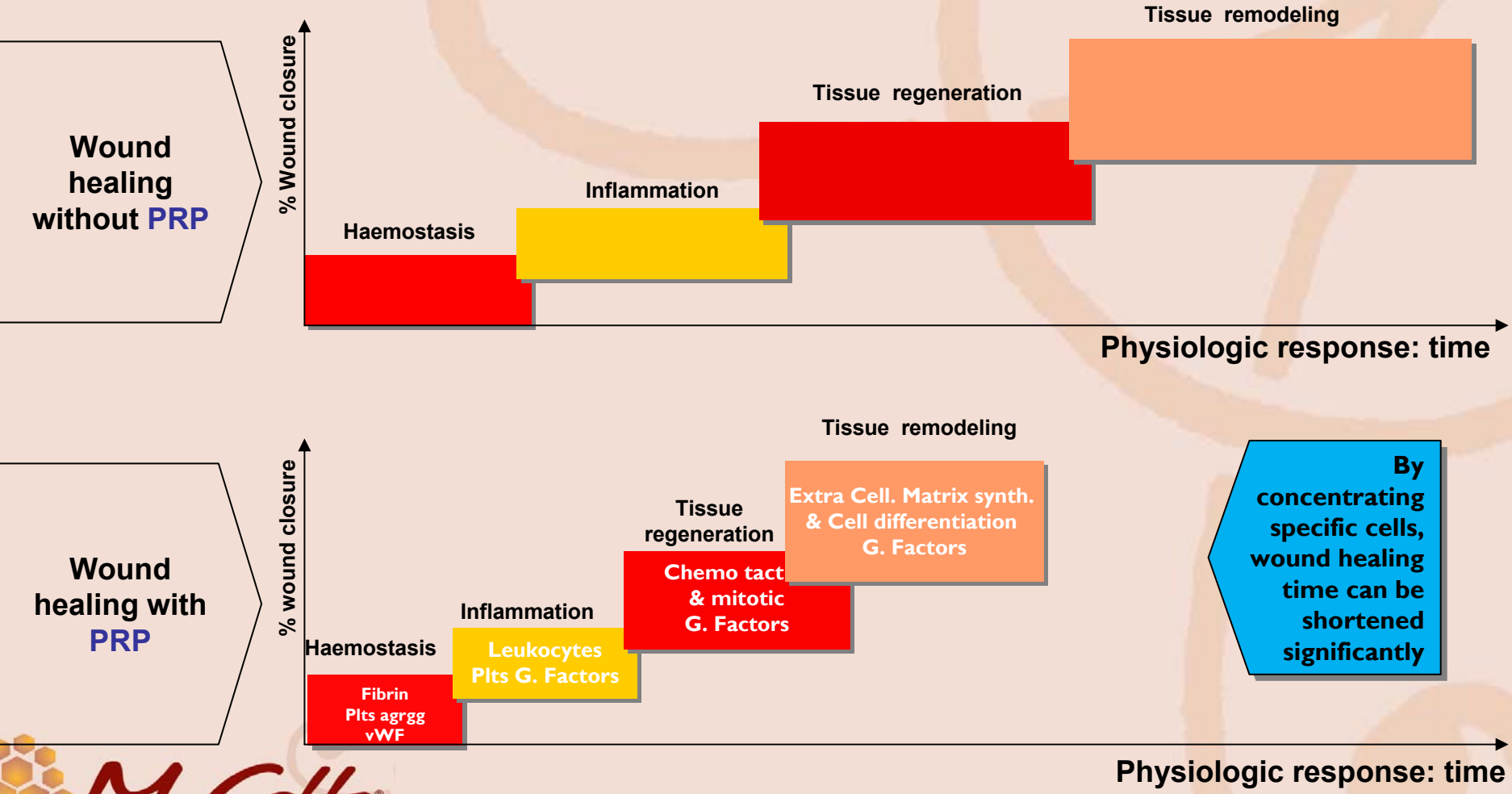

PRP
Platelets
Stem Cells
CD34+
EGF
KGF
PDGF
IL
GF
TGFβ
Stem Cells
Stem Cells
Stem Cells
Stem Cells
Stem Cells


PRP Injection
Platelet
Aggregation
& Activation
EGF
PDGF
IL
GF
TGFβ
CD34+
CD34+
CD34+
Stem Cells
Stem Cells
Stem Cells
Stem Cells
Collagen, ADP
(basic membrane)


Platelet
Activation
De-granulation
+Fibrin
+Fibronectin
+Vitronectin
EGF
PDGF
VEGF
TGFβ
Stem Cells
Angiogenesis
Collagen Production
Epithelial cells
growth
Keratinocytes,
Fibroblasts
Induction of cell
differentiation
KGF
Cells replication
Stem cells differentiation
Induction of
connective
tissue
formation
Trans Differentiation
CD34+
Chemo-
attraction of
Macrophages,
fibroblasts


Over-all Skin
Rejuvenation
Dermis & Epidermis Thickening
New Collagen Formation
Extra cellular matrix Formation
New Blood Vessels Formation
Adipse tissue formation

PRP accelerates the wound healing cascade



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PRP - advantages

- PRP Promotes local tissue growth and repair.
- Patient's safety – patient's own blood:
  - No disease transmission
  - Non toxic
  - No rejection
- Convenience – PRP prepared at doctor's office.
- Faster healing – PLT accelerate tissue synthesis.
- Cost effectiveness – No need for external substances.
- Simple and easy to use.

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PRP harvesting kits - Shortcomes

Currently, kits commonly used in the market, have the following shortcomes:

- Accidental aspiration of gel into plasma and following injections of gel into human:
  - Several reports describe that small gel particles were found floating in plasma phase, which can be visually seen with naked eye.
  - These small gel particles have been reported to cause blockage using 30G needle during the treatment while injecting plasma and $CaCl_2$ mixture.
  - As the user base expands, not all users are as careful as they should be in the preparation of PRP for injection:
    - It has been observed that needle went into gel phase during drawing PRP into syringe, in some clinics.
    - Some physicians pushed needle into gel phase during aspiration of PRP into syringe.
  - The effect of accidental injection of separation gel into human is not clear.
- Regulation problems
- Inconsistent recovery rate

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As applications of PRP will expand,

a simple and safe harvesting device of autologous PRP is mandatory.

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Presenting MyCells®:
PRP harvesting kit
By Kaylight Technologies

MyCells® kit for autologous PRP harvesting

Kit Components:

1. 1 x VACU10 (Holder attached to luer adapter attached to PSV)
2. 2 x PPT I (Vacuum gel tubes for platelet preparation) - CE 1023
3. 1 x 10ml syringe
4. 1 x Blunt needle 18Gx100mm
5. 2 x PPT I/II (Sleeve filter) - CE 1023
6. 2 x hypodermic needles 30G
7. 2 x 1ml luer lock syringe
8. 1 x hypodermic needle 21G

Additional equipment required:

Serological centrifuge (reaches 3000 RPM, Room Temp) .

MyCells® kit - Instructions for use:

1. Draw blood, into PPT I tube, containing gel & ACD
2. Centrifuge
3. Phase separation : RBC / Plasma (PRP+PPP)
4. Remove of PPP phase.
5. PRP harvesting
6. Transfer PRP into Filter sleeve to remove gel remnants
7. Inject PRP to patient in less than 5 minutes time
8. Repeat all PRP volume is consumed.

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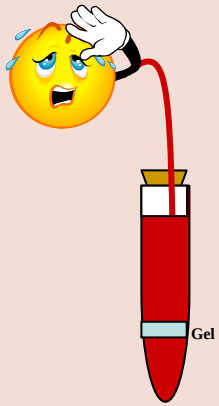
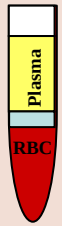
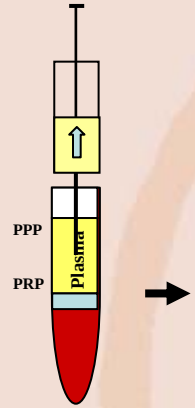


1. Draw blood, using VACU 10 (item no 1) into PPT I tube, containing gel & ACD (item no 2) (vacuum allows max 10 ml blood per tube).

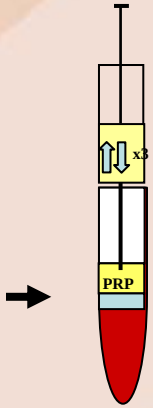
2. Centrifuge 10 min, 1200G, Room Temperature. *ACR tube plasma yield = 6-7 ml.*

Phase separation within whole blood – Plasma + Red Blood Cells

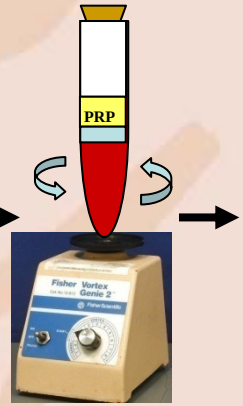




3. Removal of PPP phase. Place the tube in the rack. Attach the 10 cm blunt needle (item no 3) to 10 ml syringe (item no 4). Remove cap of tub insert 10 ml needle. Then carefully draw 50% to 60%, 3 to 3.5 ml. plasma from the surface and downward You are now removing PPP phase. Do not insert needle too deep from the surface of plasma. After drawing PPP into the syringe, discard this PPP.

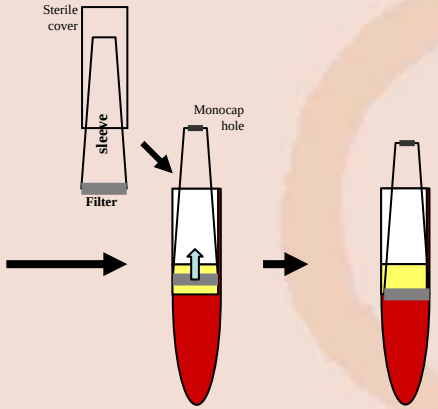


4. PRP harvesting: Using the same 10 ml syringe with 10 mm blunt needle (item no 3), you will reach to the remaining plasma. You are now dealing with PRP. Draw remaining PRP into syringe without touching the separation gel. Then gently pump back PRP in the syringe against gel surface to lift platelet on the gel surface. Carefully repeat this process 2 to 3 times. This is a very important process to harvest platelets as many as possible.

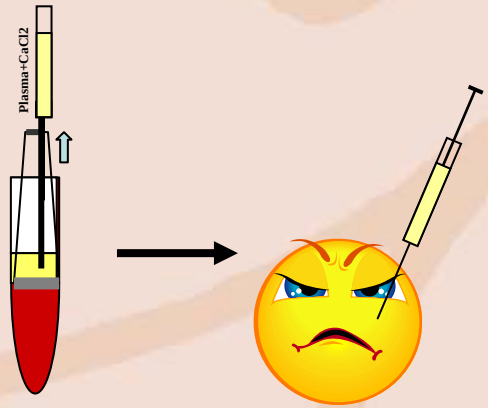


5. Cap the tube. Re-mix again PRP in remaining 2-3 ml of plasma, by gently vortexing) for 30 seconds.

PRP extraction using MyCells® kit (1)



6. Place the tube in the rack, and remove cap. Take the filter sleeve (item no 5), peel sterile cover to a half way from filter side to expose bottom side of sleeve. Hold cap with sterile cover on and insert filter into the tube until filter gently touches gel surface. PRP now enters inside of sleeve chamber.

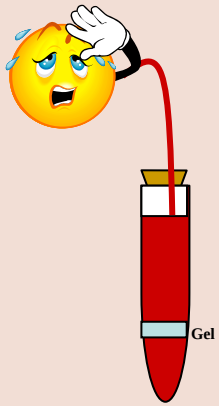


7. Draw 1 ml from the plasma in the Sleeve. Remove blunt needle from syringe and leave it in the monocap hole. Attach the 30G needle (item no 9), to the syringe.

8. Inject PRP to patient in less than 5 minutes time (otherwise a clot will develop in syringe!)

11. Repeat steps 7-8 until all PRP volume is consumed. Intra-dermal clot formation after 8 to 10 minutes.

PRP extraction using MyCells® kit (2)

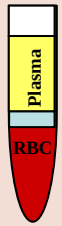


1. Draw blood, using VACU 10 (item no 1) into PPT I tube, containing gel & ACD (item no 2) (vacuum allows max 10 ml blood per tube).

1 - Prélevez le sang en utilisant le VACU 10 dans le tube contenant le gel et l'anticoagulant PPT I. Le vide permet un prélèvement de 10 ml par tube.

2. Centrifuge 10 min, 1500g, Room Temperature. *ACR tube plasma yield = 6-7 ml.*

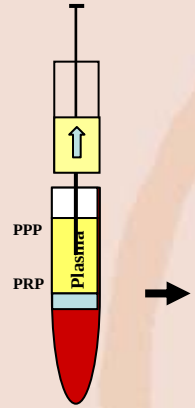
2 - Centrifugez 10 mn à 1500 g (à température ambiante). Vous obtenez 6 à 7ml de plasma dans le tube



Phase separation within whole blood – Plasma + Red Blood Cells.

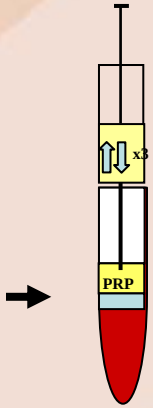
3. Remove cap from tube.

3 - Enlevez le bouchon du tube.



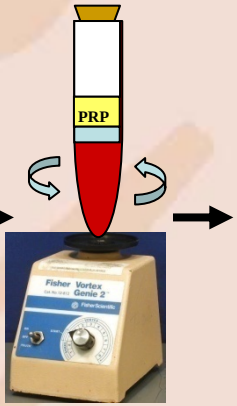
4. Removal of PPP phase. Place the tube in the rack. Attach the 100 mm blunt needle (item no 4) to 10 ml syringe (item no 3). Remove cap of tube and insert 100 mm needle until you reach to the surface of plasma. Then carefully draw plasma from the surface and downward for about 50% to 60%, 3 to 3.5 ml. You are now removing PPP phase. Do not insert needle too deep from the surface of plasma. After drawing PPP into the syringe, discard this PPP.

4 - Retirez le Plasma Pauvre en Plaquette(PPP) : Placez le tube dans le porte éprouvette. Fixez l'aiguille de 100 mm à la seringue de 10 ml. Insérez l'aiguille dans le tube jusqu'à atteindre la surface du plasma puis, retirez le plasma sur 50% à 60% (soit 3 à 3.5ml). Vous êtes en train de retirer le PPP.



5. PRP harvesting: Using the same 10 ml syringe with 100 mm blunt needle (item no 4), you will reach to the remaining plasma. You are now dealing with PRP. Draw remaining PRP into syringe without touching the separation gel. Then gently pump back PRP in the syringe against gel surface and around the tube wall to lift platelet on the gel surface. Carefully repeat this process 2 to 3 times. This is a very important process to harvest platelets as much as possible.

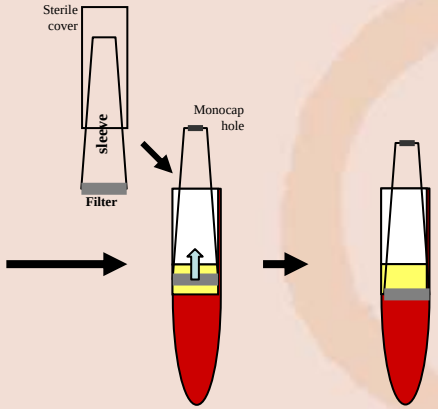
5 - Récupérez le PRP : Utilisez la même seringue de 10 ml avec l'aiguille de 100 mm. Prélevez le PRP qui reste dans la seringue sans toucher le gel de séparation. Puis doucement, redéposez le PRP dans le tube en le faisant tourner dans la main afin que le produit ruisselle sur la surface du tube et récupère ainsi le maximum de plaquettes.



6. Cap the tube. Re-mix again PRP in remaining 2-2.5 ml of plasma, by gently vortexing it (vortex speed – *number 3*) for 30 seconds.

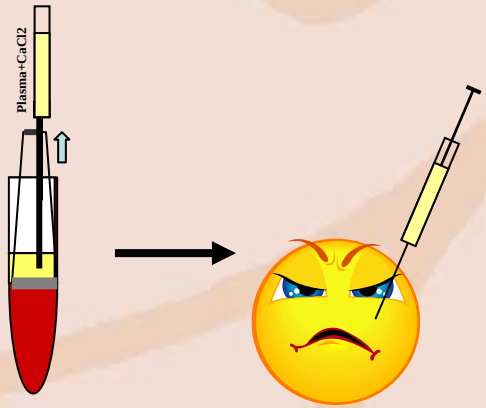
6 -Rebouchez le tube et vibrez en utilisant le Vortex doucement pendant 30 sec.

PRP extraction using MyCells® kit (1)



7. Place the tube in the rack, and remove cap from the tube. Take the filter sleeve (item no 5), peel sterile cover to a half way from filter side to expose bottom side of sleeve. Hold cap with sterile cover on and insert filter into the tube until filter gently touches gel surface. PRP now enters inside of sleeve chamber.

7 - Récupérez le PRP dans le tube filtre : Placez le tube dans le porte éprouvette, enlevez le bouchon. Prenez le tube filtre et enlevez la protection stérile sur la moitié inférieur. Tenez le haut du filtre avec l'enveloppe stérile et insérez le filtre dans le tube. Le PRP entre dans le tube filtre.



8. Draw 1 ml from the plasma in the Sleeve. Remove blunt needle from syringe and leave it in the monocap hole. Attach the 30G needle (item no 6), to the syringe.

8- Prélevez 1 ml de plasma dans la seringue. Laisser l' aiguille de 100 mm dans le trou du bouchon. Fixez l'aiguille 30g à la seringue.

9. Inject PRP to patient in less than 10 minutes time (otherwise a clot will develop in syringe!)

9- Injectez le PRP à la patiente en moins de 10 mn sinon le produit coagulera dans la seringue

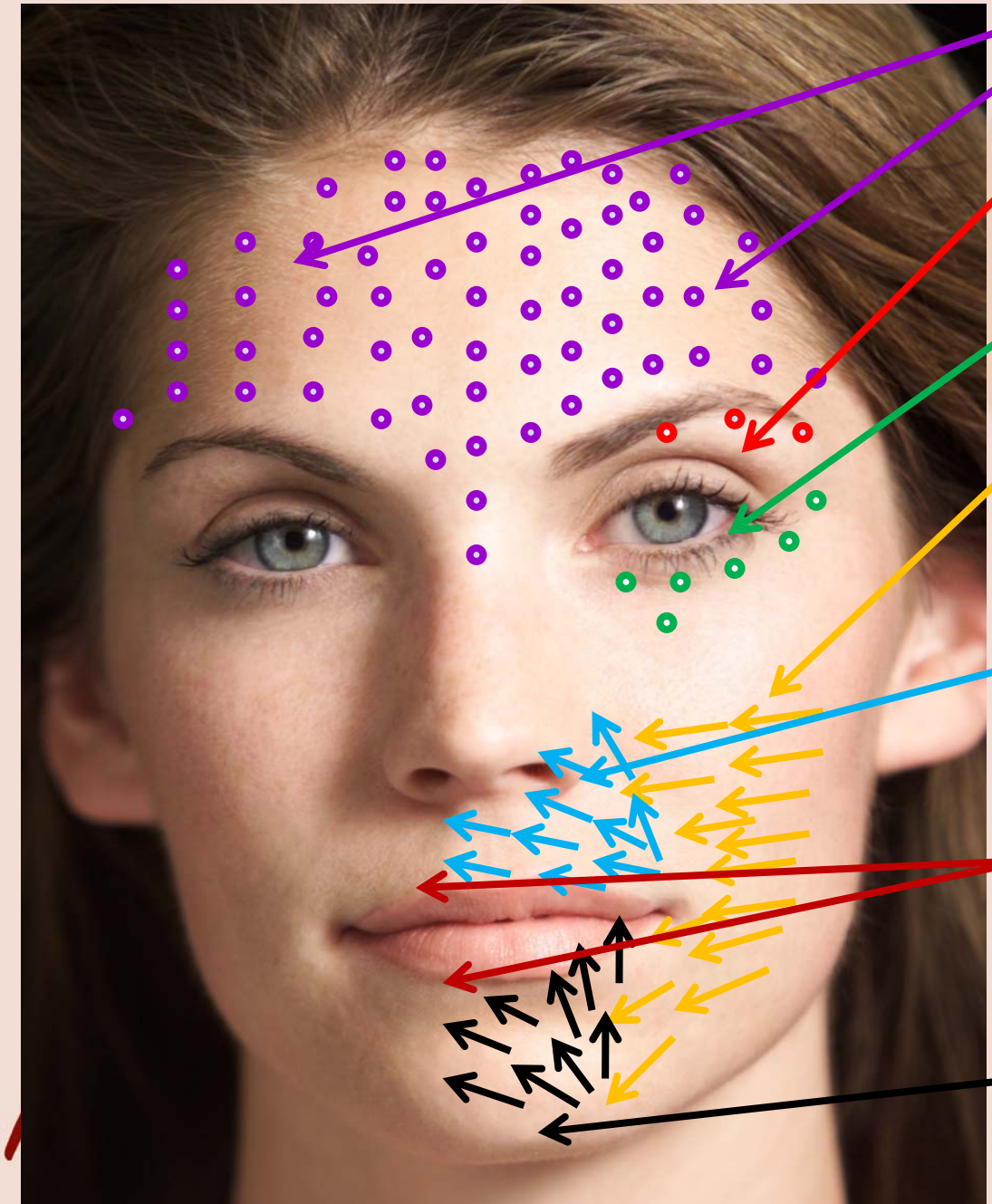
10. Repeat steps 8-9 until all PRP volume is consumed.

10.– Répétez les étapes 8-9 jusqu'à ce que tout le PRP soit utilisé.

Intra-dermal clot formation after 10 to 20 minutes.

PRP extraction using MyCells® kit (2)

MyCells® Injection Technique:



Forehead
Intradermal injections 0.05ml. Total for forehead 3ml.

Upper eyelid
Subdermal injections 0.2ml each x 3. Total 0.6ml.

Lower eyelid
Subdermal 0.2ml injections 1 cm apart. Massage evenly. Total 1-2ml.

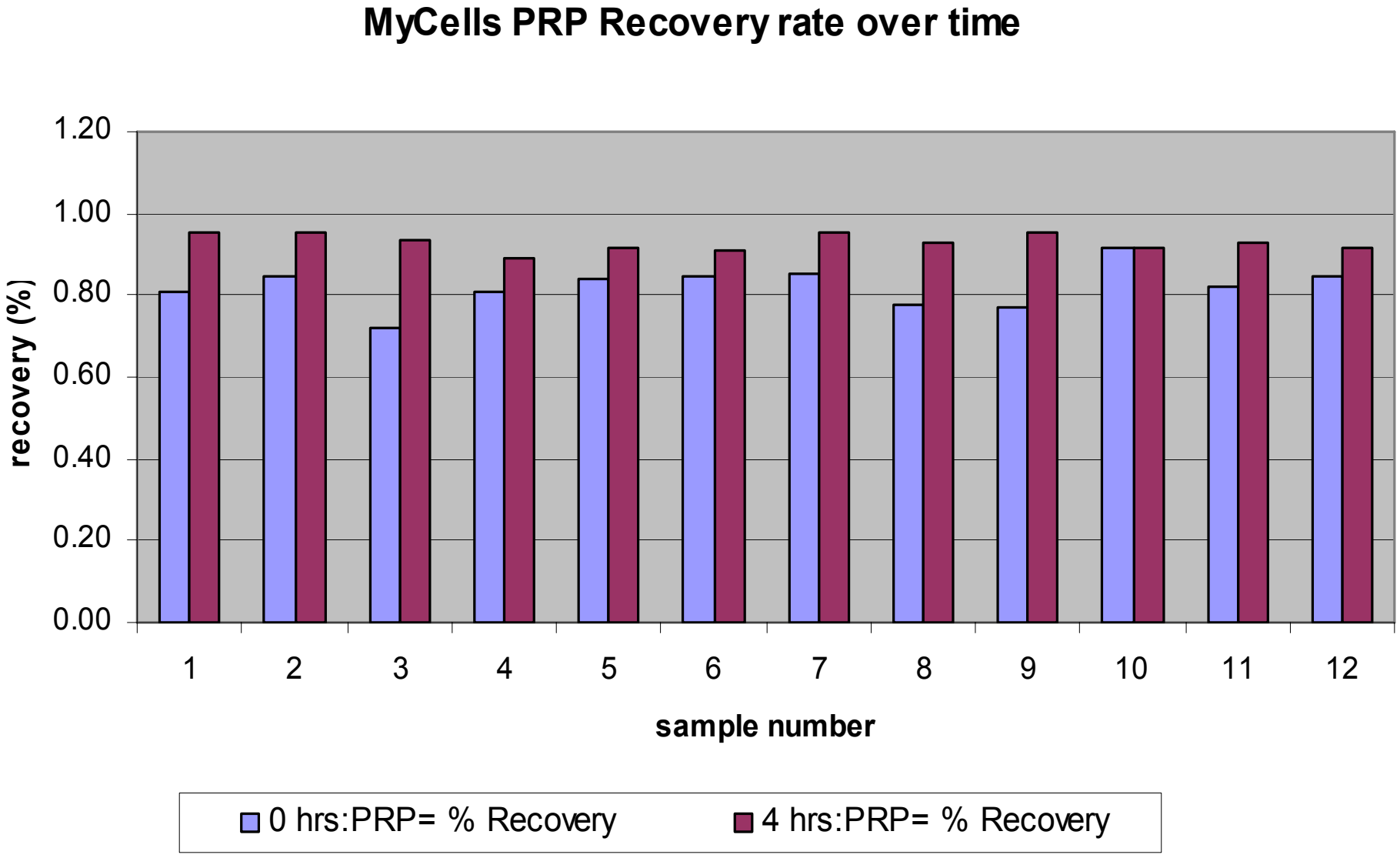
Cheeks
Subdermal & intradermal injections
Linear threading technique 0.2ml per injection. Total 3-5ml per side.

Naso-labial folds
Subdermal & intradermal injections
Linear threading technique 0.2ml per injection. Total 2-3 ml per side.

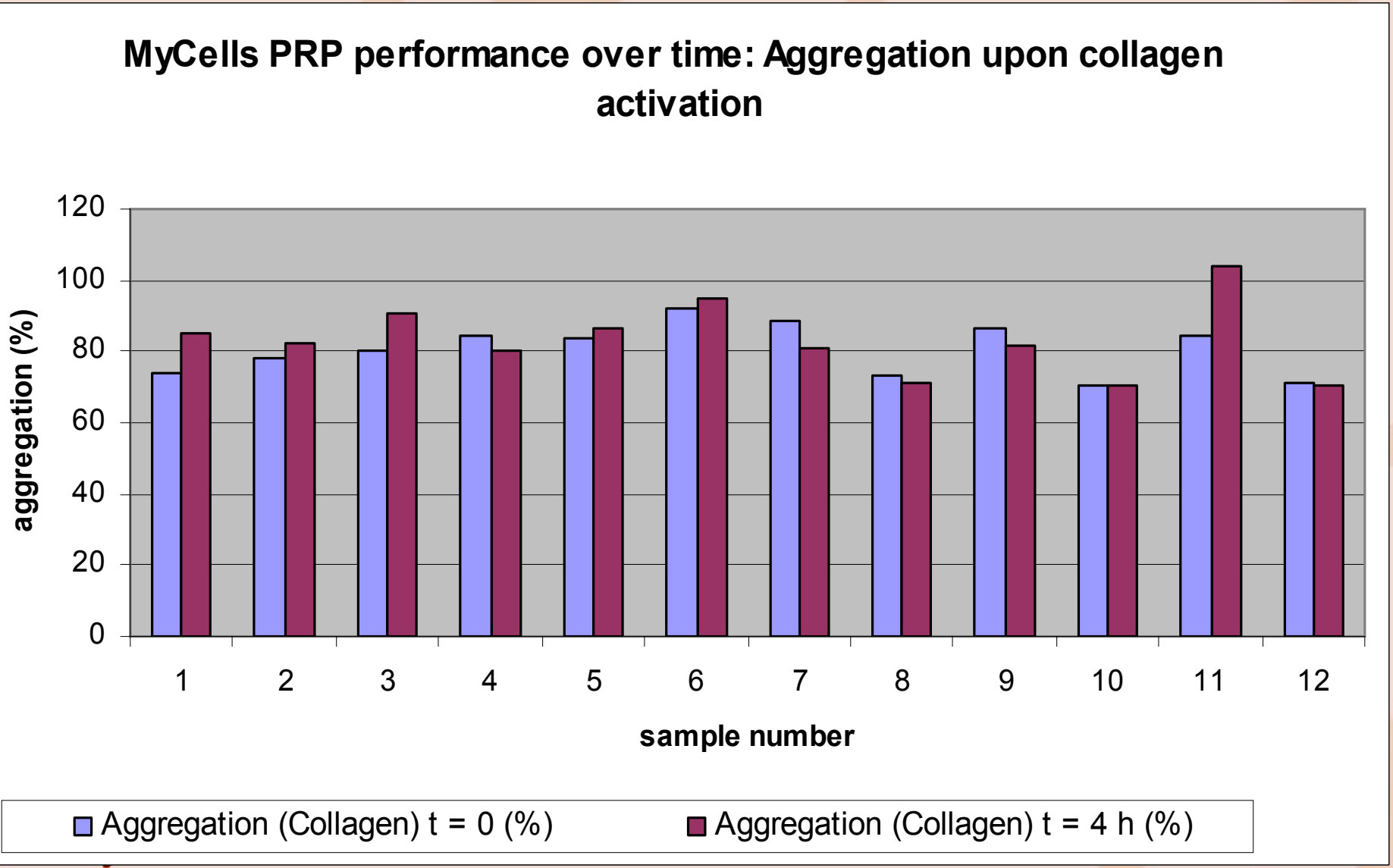
Lips
Vermillion border injections
Linear threading technique 0.2ml per injection. Total 0.4ml per quadrant.

Chin
Linear threading technique 0.2ml per injection. Total 2-3ml per side.

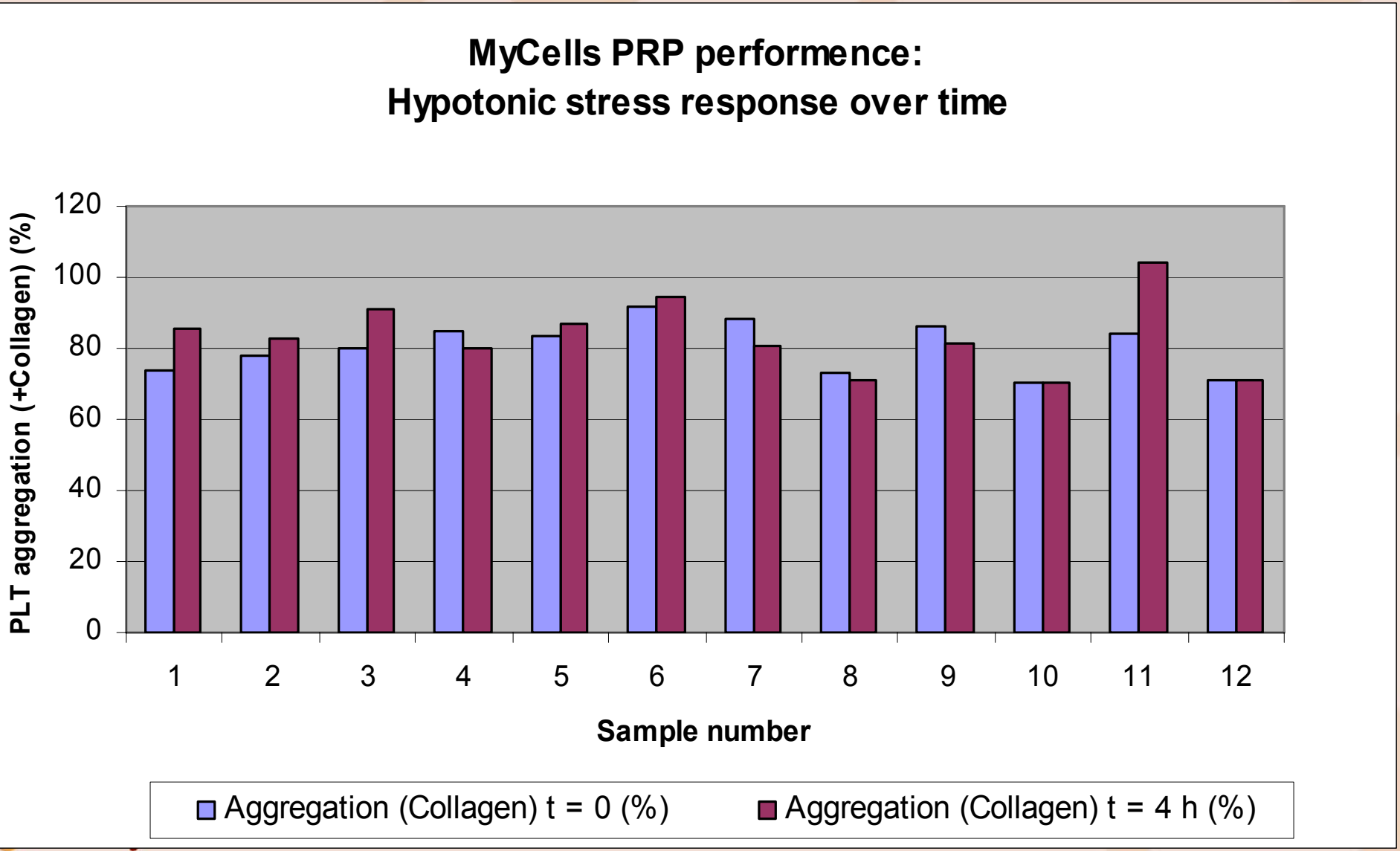
MyCells® PRP harvesting kit - Performance: Platelets integrity over time



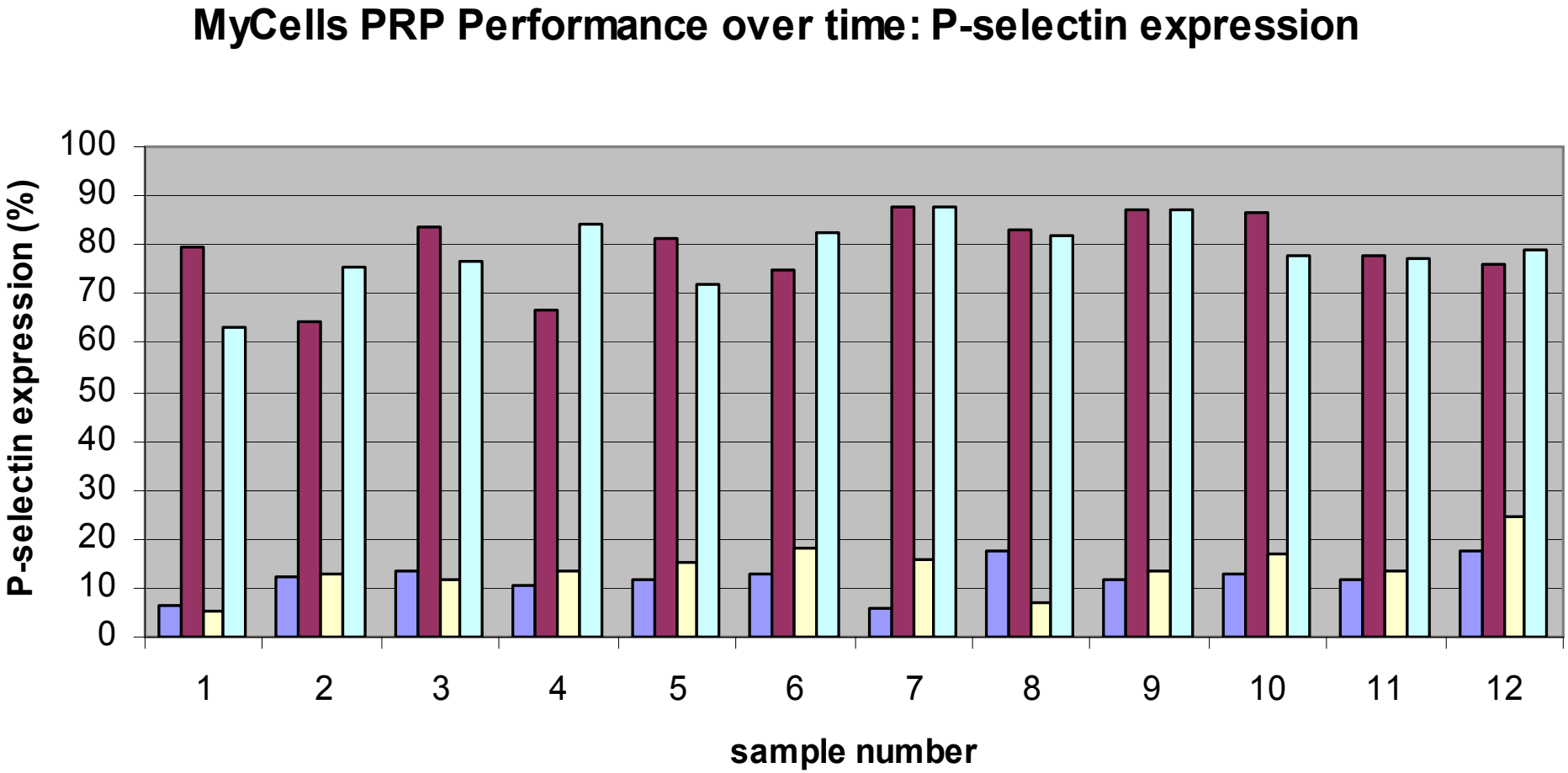
MyCells® PRP harvesting kit - Performance: Platelets integrity over time



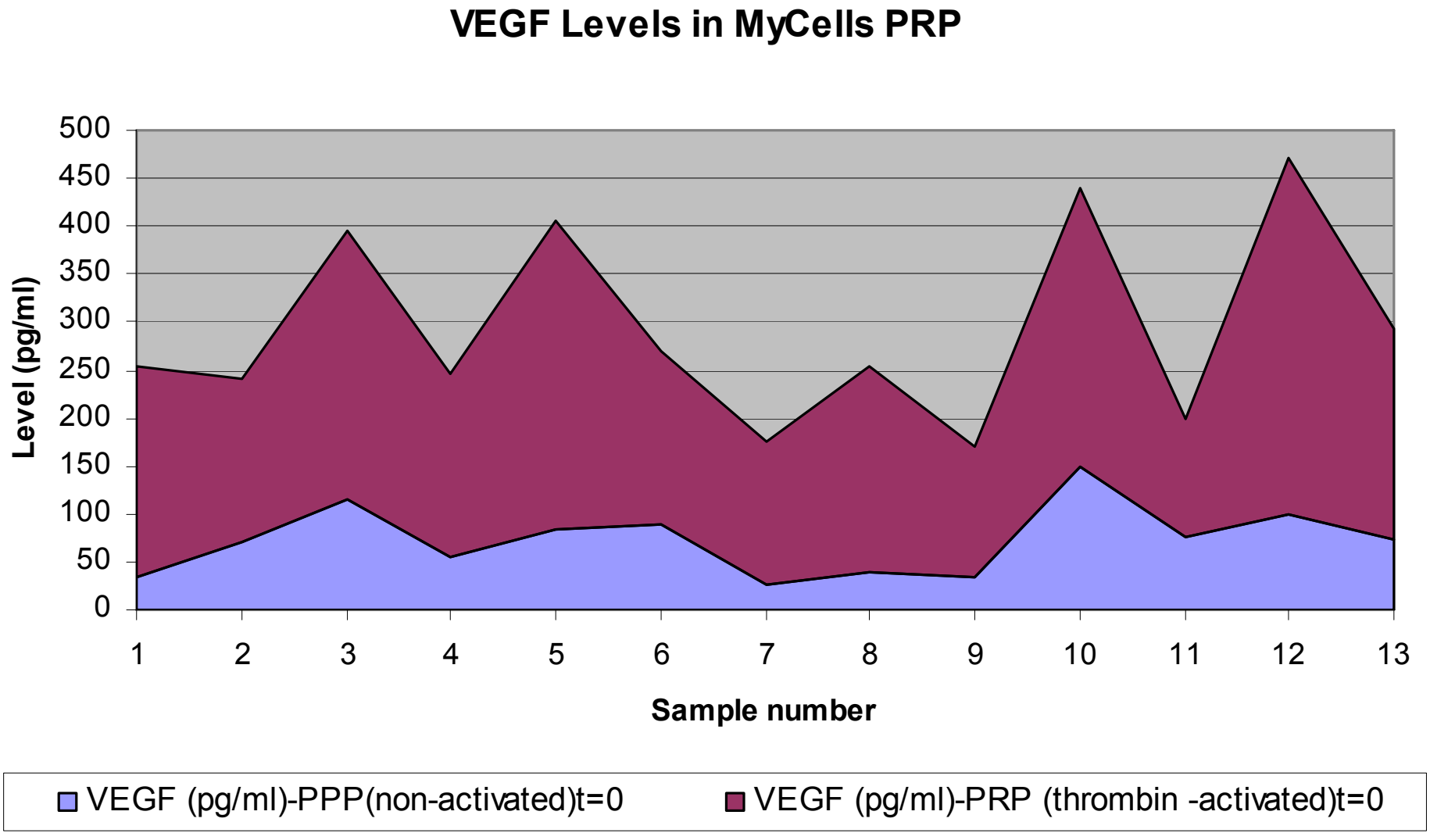
MyCells® PRP harvesting kit - Performance: Platelets integrity over time



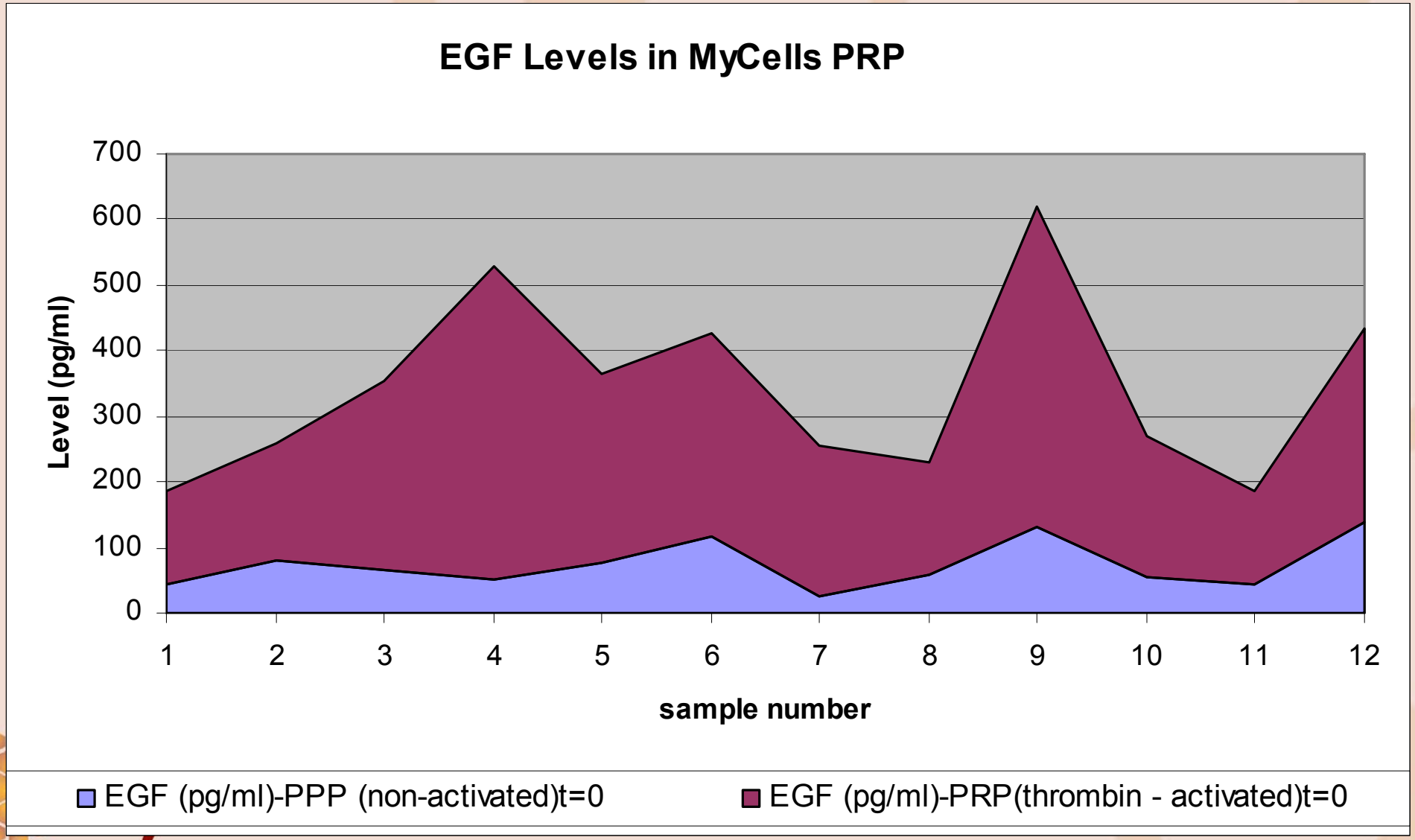
MyCells® PRP harvesting kit - Performance: Platelets integrity over time



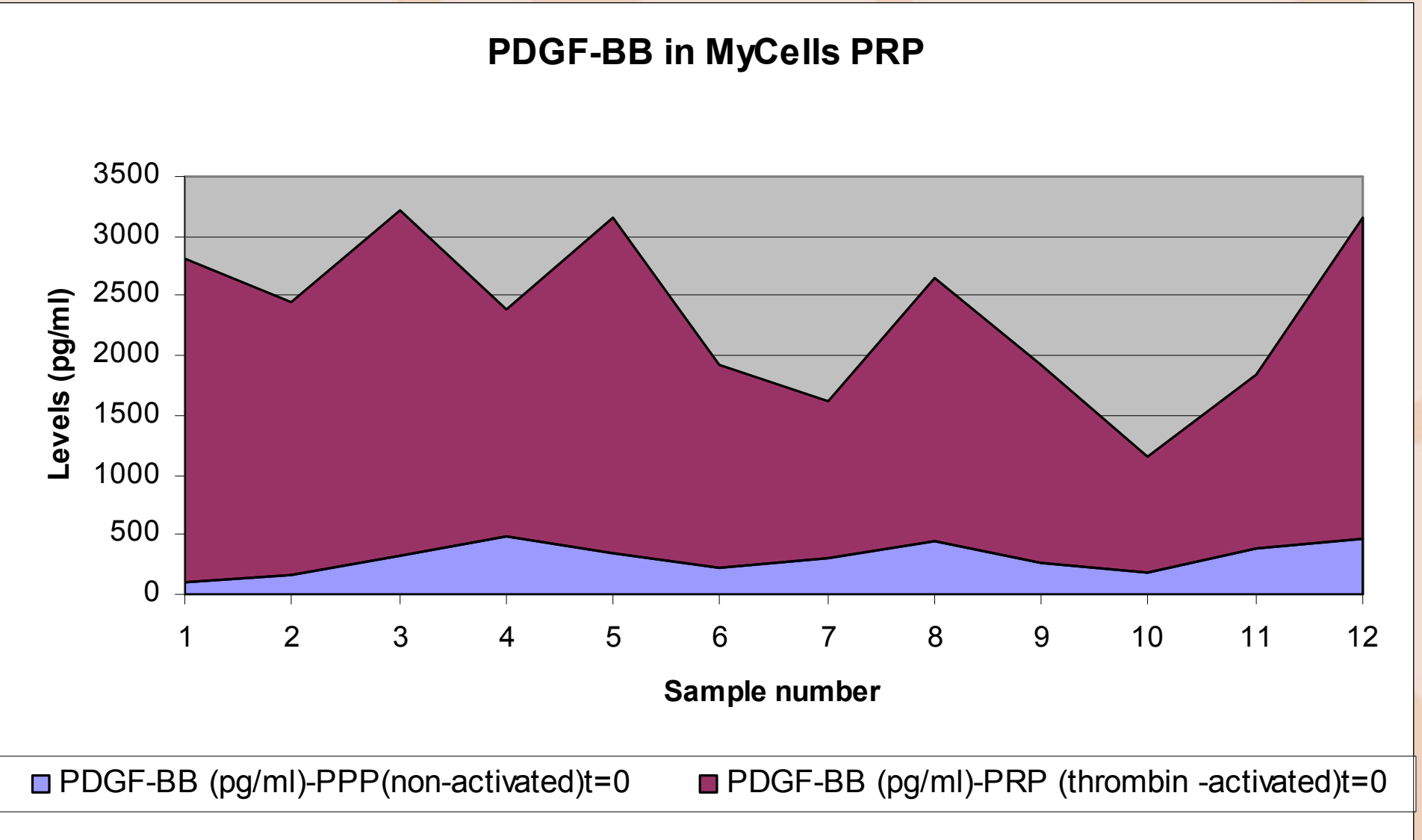
MyCells® PRP harvesting kit - Performance – Growth Factors Secretion:



MyCells® PRP harvesting kit - Performance – Growth Factors Secretion:



MyCells® PRP harvesting kit - Performance – Growth Factors Secretion:



PRP harvesting kits – Recovery rates*

Device	PRP Volume (ml)	Platelet Concentration (10^6/ml)	Platelet Recovered (%)	Platelet Enrichment (%)
Blood		275 +/- 125	100	1.0
Laboratory Centrifuge* (Anitua Protocol)	9.5 +/- 4.1	433 +/- 129	35 +/- 16	1.9
Laboratory Centrifuge* (Landsberg Protocol)	10.6 +/- 2.4	336 +/- 141	30 +/- 10	1.5
Clinaseal* Salvin Dental Specialities	7.6 +/- 1.5	401 +/- 267	39 +/- 16	1.6
ACE Surgical*	7.8 +/- 0.6	493 +/- 245	33 +/- 10	1.8
AG Curasan*	7.6 +/- 1.6	344 +/- 192	29 +/- 14	1.4
3i PCCS*	7.0 +/- 1.5	939 +/- 284	61 +/- 9	3.2
Harvest Technologies "SmartPReP"	7.4 +/- 0.5	1086 +/- 227	62 +/- 4	4.0
MyCells kit	3.34 +/- 0.6	546.7 +/- 273	**87.5 +/-9**	2.4

* PRP- Evidence to support its use, Marx R E, J. Oral Maxillofac Surg 62: 489, 2004

MyCells® PRP harvesting kit - Performance:

Presence of Progenitor $CD34^+$ Stem Cells in MyCells PRP:

- 10 ml Venous blood sample:
  1.6×10^9 $CD34^+$ stem cells
- 6 ml post centrifugation MyCells PRP :
  1.2×10^9 $CD34^+$ cells

- **= 80% Recovery**

MyCells® PRP harvesting kit - Advantages:

- ✓ Safety in use – no accidental aspiration of gel into plasma and following injections of gel into human.
- ✓ High Recovery rate (>85%)
- ✓ Larger blood volume in collection tube (10 ml)
- ✓ Regulation: ISO-13485, CE, FDA.

MyCells® harvesting kit
Current Regulatory Status

- All Kaylight PRP harvesting kits components have been approved by the following regulatory bodies:
  - **ISO 13485 (Nov 07).**
  - **CE *Class IIA* 1023 for *in vivo* usage for re-injection (Feb 08).**
  - **FDA 21-CFR-880.5860 (Sep 09).**

MyCells®

Kaylight Technologies:

Producer:

- Vacuum PRP harvesting Tubes and all kit components (except $CaCl_2$) will be produced by Kaylight Technologies Ltd, Holon, Israel.

Previous Experience with PRP

- In the dentistry field

Certificates:

Kaylight Technologies Ltd, Holon, Israel holds the following quality certificates:

- ISO-9001/9002
- ISO 13485
- GMP Certificate
- Free Sell Certificate
- CE – *In Vitro* Diagnosis
- CE – Medical Devices for kit accessories
- CE 1023 – *In vivo* usage for re-injection in humans
- FDA 21-CFR-880.5860 for marketing in the USA

Remarks:

- All syringes in the kit are sterile, pyrogen and latex free.
- All tubes and filters undergo gamma irradiation.
- Each batch of tubes specifically undergo tests for sterility and pyrogenicity.

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MyCells® PRP harvesting kit - Quality Tests :

MyCells® PRP harvesting kits underwent the most stringent quality tests by:

Harlan Biotech Ltd, Rehovot, Israel

and

BSL Bioservice, Laboratories GmbH, Planegg, Germany

The Tests:

- Acute toxicity in mouse
- Delayed-type hypersensitivity (Guinea Pig Maximisation Test)
- In Vitro Cytotoxicity Screening Assay:
- Bioburden estimation
- Bacterial endotoxins test
- In vitro Hemolysis Test under static Conditions (Hemolysis potential test)
- Analysis of the Partial (PTT) & Activated Partial (aPTT) Thromboplastin (coagulation) Time

MyCells®

MyCells® harvesting kit Components - detailed:

Tubes:

2 x 10 ml Vacuum Blood Collection*, Haemo-repellent glass tube for separation of PRP.

Gel:

Proprietary Z-Gel.

Anti Coagulant:

ACD (Adenine Citrate Dextrose) solution containing 1 ml 0.109 M liquid Buffer of Sodium Citrate + Dextrose, based on water solution.

Filtered sleeve:

Proprietary Filtered sleeve, with pores size of 10µM.

**The tube fits every standard centrifuge for blood separation (with swing rotor).*

MyCells®

MyCells®: Academic Research

TEL AVIV UNIVERSITY אוניברסיטת תל-אביב

The Chaim Sheba Medical Center at Tel Hashomer

Evaluation of the molecular mechanism underlying the regenerating effects of MyCells®.

Rima Dardik, Ph.D, Head, Unit of Genetic Diagnosis of Hemophilia, Institute of Thrombosis and Hemostasis, Sheba Medical Center, Affiliated to Tel Aviv University, Israel, and **Livia Theodor**, Ph.D, MBA, MyCells®, Israel

MyCells®

Introduction

Activated platelets release:

- adhesive glycoproteins
- growth factors

Following subcutaneous injection, these proteins and GF interact with cells residing in the subcutaneous tissues. eg:

- skin fibroblasts
- endothelial cells,
- osteoblasts.

Upon binding to their cellular receptors, glycoproteins and growth factors activate intracellular signaling events, mediating:

- Angiogenesis
- cell proliferation,
- migration,
- survival
- production of extracellular matrix proteins.

MyCells®

Introduction (2)

- In view of the crucial role of angiogenesis in wound healing and tissue regeneration, we examined the effect of activated platelets on the expression of 84 genes involved in positive and negative regulation of angiogenesis in endothelial cells, using a specialized real-time PCR array.

- Platelet activation was performed by thrombin-receptor activating peptide (TRAP).

Results

Proangiogenic growth factors	Upregulation (x fold)
Transforming growth factor, alpha	**2.67**
Transforming growth factor, beta 1	**2.59**
Vascular endothelial growth factor A	**3.04**
Hypoxia-inducible factor 1, alpha subunit - regulator of VEGF expression	**6.29**
Fibroblast growth factor 2 (basic)	**13.02**
Cell Adhesion Receptors	**Upregulation (x fold)**
Integrin, alpha V	**3.16**
Integrin, beta 3	**3.17**
Laminin receptor, alpha 5	**7.56**
Endoglin	**4.05**

MyCells®

Conclusions

Our preliminary results indicate that:

Exposure of endothelial cells to activated platelets induces enhanced expression of:

- TGF –alpha,
- TGF- beta,
- VEGF (Vascular endothelial GF)
- HIF (regulator of VEGF)
- basic FGF

growth factors involved in regulation of angiogenesis, wound healing and tissue regeneration

- Integrin alpha v
- Integrin beta 3
- Laminin receptor

Factors involved in cell adhesion, survival, migration and regulation of growth factor receptor activity. All these processes play important roles in angiogenesis, tissue regeneration and cell adhesion to the extracellular matrix proteins.

MyCells®


THANK YOU