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

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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

All theses lead to:
- Changes in skin texture, firmness, radiance, volume, flexibility and wrinkle formation
What happens when we get older?

24 years old

48 years old
Current Solutions for Skin Rejuvenation:

DERMO COSMETICIS

FILLERS

Renewal of the corneal layer

Germinative layer Stimulation

Dermis Stimulation

Hyperpigmentation removal

Wrinkles & volumes correction

Plastic Surgery
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.
Presenting MyCells®:
Bio stimulation with Platelet Rich plasma (PRP)

Architectural skin reconstruction/reorganization
MyCells® – a revolutionary method allowing rejuvenation of the skin, using the patient’s own (autologous) cells, with long lasting effect.
MyCells® PRP harvesting kit - Visible Results:

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

MyCells® PRP harvesting kit - Visible Results:

Aged 60: 21 months after 1 injx.
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.
MyCells® PRP harvesting kit - Visible Results:

54 years: 2 months after 1 injx.
MyCells® PRP harvesting kit -
Visible Results:

46 years: Acne scars - 3 months after 2 injx.
MyCells® PRP harvesting kit -
Visible Results:

51 years: 2 months after 1 injx
MyCells® PRP harvesting kit - Visible Results:

54 years: 2 months after 2 injx.
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.
My Cells® 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.
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).
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...

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

5. Stem cells differentiation and tissue formations

(In addition ECM like fibronectin, vitronectin, thrombospondin...)

Platelets & Megakaryocytes vol.2 Dr. J. Gibbins, M. Mahaut-Smith
Key Mediators in Platelet Adhesion, Activation and Aggregation

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
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
What do PLT do at the wound site?

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

Platelet Aggregation & Activation

Collagen, ADP (basic membrane)

Stem Cells

EGF

ILGF

TGFβ

PDGF

CD34+
Platelet Activation → De-granulation

+ Fibrin + Fibronectin + Vitronectin

Cells replication
Stem cells differentiation

Epithelial cells growth
Stem Cells

Keratinocytes, Fibroblasts

Angiogenesis

Collagen Production

Induction of connective tissue formation
Stem Cells

Induction of cell differentiation
Stem Cells

Chemo-attraction of Macrophages, fibroblasts

EGF

PDGF

VEGF

TGF-β

FGF

Stem Cells

CD34+

CD34−
Over-all Skin Rejuvenation

Dermis & Epidermis Thickening

Extra cellular matrix Formation

New Collagen Formation

New Blood Vessel Formation

Adipose tissue formation
PRP accelerates the wound healing cascade

Wound healing with PRP

By concentrating specific cells, wound healing time can be shortened significantly.

Physiologic response: time

% Wound closure

Wound healing without PRP

Tissue remodeling

Tissue regeneration

Fibrin
Plts agrgg
vWF

Leukocytes
Plts G. Factors

Chemo tactic
& mitotic
G. Factors

Extra Cell. Matrix synth.
& Cell differentiation
G. Factors

Haemostasis

Inflammation

Tissue regeneration

% wound closure

Physiologic response: time

By concentrating specific cells, wound healing time can be shortened significantly.

My Cells®
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.
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 CaCl₂ 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
As applications of PRP will expand, a simple and safe harvesting device of autologous PRP is mandatory.
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.
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. 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.
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).

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

3. Remove cap from tube.


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 into 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.

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.
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.

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.

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

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

Intra-dermal clot formation after 10 to 20 minutes.
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 Recovery rate over time

Sample number:

- 0 hrs:PRP (% Recovery)
- 4 hrs:PRP (% Recovery)
MyCells® PRP harvesting kit - Performance: Platelets integrity over time

MyCells PRP performance over time: Aggregation upon collagen activation

- Aggregation (Collagen) t = 0 (%)
- Aggregation (Collagen) t = 4 h (%)
MyCells® PRP harvesting kit - Performance: Platelets integrity over time

MyCells PRP performance:
Hypotonic stress response over time

Sample number

PLT aggregation (+Collagen) (%)

0 20 40 60 80 100 120

Aggregation (Collagen) t = 0 (%)  Aggregation (Collagen) t = 4 h (%)
MyCells® PRP harvesting kit - Performance: Platelets integrity over time

MyCells PRP Performance over time: P-selectin expression

- P-selectin expression t=0, resting (%)
- P-selectin expression t=0, ADP (%)
- P-selectin expression t=4h, resting (%)
- P-selectin expression t=4h, ADP (%)

Sample number:

1 2 3 4 5 6 7 8 9 10 11 12
MyCells® PRP harvesting kit - Performance – Growth Factors Secretion:

VEGF Levels in MyCells PRP

![Graph showing VEGF levels in MyCells PRP](graph.png)

- **VEGF (pg/ml)-PPP (non-activated) t=0**
- **VEGF (pg/ml)-PRP (thrombin-activated) t=0**
MyCells® PRP harvesting kit - Performance – Growth Factors Secretion:

EGF Levels in MyCells PRP

- EGF (pg/ml)-PPP (non-activated) t=0
- EGF (pg/ml)-PRP (thrombin - activated) t=0

Sample number

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

MyCells® PRP harvesting kit - Performance:

Presence of Progenitor CD34⁺ Stem Cells in MyCells PRP:

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

= 80% Recovery
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).
Kaylight Technologies:

**Producer:**
- Vacuum PRP harvesting Tubes and all kit components (except CaCl₂) 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.
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® 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®: Academic Research
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
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.
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

<table>
<thead>
<tr>
<th>Proangiogenic growth factors</th>
<th>Upregulation (x fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transforming growth factor, alpha</td>
<td>2.67</td>
</tr>
<tr>
<td>Transforming growth factor, beta 1</td>
<td>2.59</td>
</tr>
<tr>
<td>Vascular endothelial growth factor A</td>
<td>3.04</td>
</tr>
<tr>
<td>Hypoxia-inducible factor 1, alpha subunit — regulator of VEGF expression</td>
<td>6.29</td>
</tr>
<tr>
<td>Fibroblast growth factor 2 (basic)</td>
<td>13.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Adhesion Receptors</th>
<th>Upregulation (x fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Integrin, alpha V</td>
<td>3.16</td>
</tr>
<tr>
<td>Integrin, beta 3</td>
<td>3.17</td>
</tr>
<tr>
<td>Laminin receptor, alpha 5</td>
<td>7.56</td>
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<tr>
<td>Endoglin</td>
<td>4.05</td>
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</tbody>
</table>
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

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.

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