PRP update: What have we learned?

Dr José Fábio Lana
Summary

• PRP – definition and controversies
• Collecting and obtaining ➔ different types
• PRP contents
• How we do it?
• Proposal of a new classification : MARSPILL
Introduction

PRP

Platelets concentrated in small volume of plasma

Autologous non-immunogenic therapy

Contains a high concentration of growth factors (GFs) and cytokines
Introduction

• PRP activation bursts the release of platelets α-granules rich in proteins and growth factors:
  – Platelet-derived growth factor (PDGF)
  – β- transforming growth factor (TGF-β)
  – Insulin-like growth factor (IGF-1)
  – Vascular endothelial growth factor (VEGF)
  – Epidermal growth factor (EGF).
Introduction

• Increasing number of research and its extensive use in diverse areas
• There is no consensus regarding the classification used for different types of PRP obtained via machine or in house
• Different terminologies may be observed for the same type of PRP and vice versa
Collecting and obtaining PRP
Peripheral blood harvest → Centrifugation → PRP

- Plasma fraction collected
- Activation

Time Force ≠
• Different classifications and the lack of communication in the literature contributed to the emergence of different terminologies.

• Besides, the cellular and molecular components present in the PRP has not been stated and fully analyzed in the current ratings, which compromises its reproducibility and better understanding regarding the biological effectiveness.
PRP Contents
PRP Contents

• Consists mainly of cellular and molecular components:
  
  – **Cellular components**: platelets and peripheral blood mononuclear cells (PBMCs)
  
  – **Molecular components**: growth factors and a diverse family of immunomodulatory proteins
Cellular Components
Cellular Components

Platelets

- Cytoplasmic anucleated fragments derived from megakaryocyte
- Can be activated with agonists or even during the centrifugation procedure
- Release their α-granules rich in low molecular weight proteins such as GFs and cytokines

Mononuclear Cells

- Neutrophils
- Monocytes
- T and B Lymphocytes
- Natural Killers (NK) cells
- Stem and progenitor cells
Buffy coat

(a) Neutrophil: Multilobed nucleus, pale red and blue cytoplasmic granules
(b) Eosinophil: Bilobed nucleus, red cytoplasmic granules
(c) Basophil: Bilobed nucleus, purplish-black cytoplasmic granules
(d) Lymphocyte (small): Large spherical nucleus, thin rim of pale blue cytoplasm
(e) Monocyte: Kidney-shaped nucleus, abundant pale blue cytoplasm
Mononuclear cells

- Neutrophils
  - Release TGF-β, VEGF and PDGF
  - + GFs in PRP
    - Angiogenesis
    - Vasculogenesis

- TNF-α
  - Macrophage Inflammatory Protein (MIP-α)
  - Recruit Monocytes
Mononuclear cells

- Monocytes
- Macrophages
- Dendritic Cells
Macrofages

- Special attention due to its plasticity
- Receive endogenous and exogenous signals → repair or replace effective cells and intercellular matrices
- Usefully modulating innate immunity against a variety of conditions ranging from cancer to atherosclerosis
- M1 → promotes host defense
- M2 → heal function
- After injury: M1 → M2
Macrophages

• Secret:
  – bone morphogenetic proteins (BMPs)
  – Interleukin-1β (IL-1β)
  – FGF, TGF-β, PDGF and IGF

• Promote the necessary recruitment and proliferation of osteoblasts, stem cells and progenitor cells
Macrophages

- Monocyte
  - GM+IL-4
  - MCP1
  - GM-CSF
  - M-CSF
- M1 macrophage
- M2 macrophage
- Pre-osteoclast
- Osteoclast
- Macrophage foam cell

Transformations:
- Monocyte → M1 Macrophage
- Monocyte → M2 Macrophage

M1 Macrophage:
- TGF-β
- IL-1
- IL-6

M2 Macrophage:
- IL-4
- IL-10
- IFN-γ

Functions:
- Acute Inflammation
- Inflammatory Mφ
- Resolving Inflammation
- Pro-resolving Mφ

Pathogen:
- NOD1
- NOD2
- RhoA
- NADPH oxidase
- INFγ
- TNFα

Increased bactericidal activity
- Increased pro-inflammatory cytokines
- Efferocytosis low

Decreased bactericidal activity
- Increased anti-inflammatory cytokines
- Efferocytosis high
Macrophages
Lymphocytes

1. B lymphocytes
2. T lymphocytes
3. T helper

Functions

- Produce antibodies
- Activate other cells
- Lyses foreign organisms
Stem and Progenitor Cells

1. Hematopoietic Stem Cells (HSCs)
   ✓ Auto renew ability

2. Hematopoietic Progenitor Cells (HPCs)
   ✓ Pluripotency

3. Endothelial Progenitor Cells (EPCs)
   ✓ Neovasculogenesis and maintenance of vascular integrity
Stem and Progenitor Cells

- **Mesenchymal stem cells (MSCs)** are also found in the peripheral circulation → occurrence of lesions or inflammatory processes

- It is assumed that by injuries in different parts of the body, MSCs are recruited to these sites through mobile signals to compose the niche cells involved in the regeneration process and/or tissue repair (no evidence)
ACTIVATED PLATELETS ARE CRITICAL TO RECRUITING STEM CELLS BONE MARROW CELLS

SDF-1 - recruits progenitor cells for tissue regeneration

VEGF - critical to vasculogenesis

Dr. C. David B. M. Harrell, OF, FRIPH
Molecular Components
Molecular Components

- Peptides and proteins released by the platelet α-granules
  - Growth factors and cytokines

- PDGF
- TGF-β
- IGF
- VEGF
- EGF

Ability to regulate the migration and cell proliferation and play a major important role in the initial healing process
Growth Factors

- **PDGF**
  - regulates the migration of macrophages
  - attracted them to the site of injury
  - plays a role in chemotaxis and angiogenesis.

- **TGF-β**
  - stimulates the proliferation of osteoblasts
  - Regulates collagen deposition in bone healing and scarring of tissue

- **IGF**
  - stimulates the proliferation of osteoblasts
  - Regulates collagen deposition in bone healing and scarring of tissue
Growth Factors

- **VEGF**
  - acts on angiogenesis
  - acts on endothelial cell proliferation

- **EGF**
  - acts on endothelial cells and fibroblasts
  - stimulates angiogenesis, cellular proliferation and synthesis of collagen and epithelial tissue.
Growth Factors
Cytokines

- Comprise a large and diverse family of immunomodulatory proteins such as interleukins (IL-1, IL-4, IL-6 and IL-10), interferons, fibrin, fibronectin and vitronectin

- These molecules send various stimulatory signals, modulatory or inhibitory to the different cells of the immune system acting in the cell itself (autocrine) in nearby cells (paracrine) and at distance (endocrine)
# Cytokines

<table>
<thead>
<tr>
<th>Pro-inflammatory</th>
<th>Anti-inflammatory</th>
<th>Multifunctional</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Th1-type,</strong> stimulatory</td>
<td><strong>Th2-type,</strong> inhibitory</td>
<td></td>
</tr>
<tr>
<td>IFN$_\gamma$</td>
<td>IL-4, IL-10, IL-1ra, IL-2</td>
<td>IL3, IL-1$\beta$</td>
</tr>
<tr>
<td>TNF$_\alpha$</td>
<td>TNFsr</td>
<td>MCP-1</td>
</tr>
<tr>
<td>IP-10</td>
<td>TGF-β2</td>
<td>sCD40L</td>
</tr>
<tr>
<td>IL-2, IL-6, IL-8,</td>
<td></td>
<td>Growth factors:</td>
</tr>
<tr>
<td>IL-12, IL-17</td>
<td></td>
<td>IL3 and G-CSF</td>
</tr>
</tbody>
</table>
Cytokines
Interleukins

- Source: monocytes, macrophages, and B and T lymphocytes
- Stimulation of CD4+ cells, proliferation and activation of B lymphocytes, neutrophils, monocytes and macrophages.

- Produced by B and T lymphocytes and primarily monocytes.

Proinflammatory effects
Interleukins

- Synthesized by Th2 cells
- Determine the profile of the immune response and increase the synthesis of MHC-II

Anti-inflammatory effects

- Produced by CD8+ cells, B lymphocytes, mast cells and monocytes
- Inhibit the synthesis of other cytokines
How do we do it?
Peripheral blood is collected in sterile syringes containing heparin.

The blood is placed into the sterile tubes.

After first centrifugation at 300 x g for 5 minutes, red cells are deposited into the bottom and only the supernatant will be collected.
Collecting the supernatant and placing into another tubes.

After second centrifugation at 700 x g for 17 minutes, 80% represents PPP (which will be placed in a another tube) and the 20% left represent the PRP, which will be homogenized.

The PRP is homogenized and placed into a syringe ready to be applied.
PLATELET RICH PLASMA - PRP – IOC PROCEDURE

PRP ready to be applied

With the assistance of ultrasound, the exact place to be applied is found

Application guided by ultrasound
Proposal of a new PRP classification: MARSPILL
MARSPILL Classification

Due to the biological efficacy of mononuclear cells in local tissue regeneration, it is proposed to guide the classification of PRP according to its concentration from the values presented in whole blood and including HSCs and cells of immunity already described.

Platelet Rich Plasma and RICH Mononuclear cells (PRP- RMC)

Platelet Rich Plasma and POOR Mononuclear cells (PRP- PMC)
MARSPILL Classification

In this classification, will be considered:
MARSPILL Classification

- **Method**: Handmade (H) Machine (M)
- **Activation**: Activated (A+) Not Activated (A-)
- **Red Blood Cells**: Rich (RBC-R) Poor (RBC-P)
- **Spin**: One Spin (Sp1) Two spins
- **Platelet Number**: PL 2-3 / PL 4-6 PL 6-8
- **Image Guided**: Guided (G+) Not Guided (G-)
- **Leukocytes Concentration**: Rich (Lc-R) Poor (Lc-P)
- **Light Activation**: Activated (A+) Not Activated (A-)
MARSPILL Classification

Example of a PRP production of clinical study through MARSPILL classification:

<table>
<thead>
<tr>
<th>Standardized PRP according the New Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP-RMC (Platelet Rich Plasma, Rich in Mononuclear Cells)</td>
</tr>
</tbody>
</table>

- **M (H)**, A (A-), R (RBC-P), S (Sp²), P (PL[4-6]), I (G+), L (Lc-R[2-3]), L (L-)
Conclusions

• There is no standardization of nomenclature used in the literature

• Consequently, different terminologies may be observed for the same type of PRP

• This new classification focus on mononuclear cells that are so important as platelet content
Conclusions

• Progenitor cells are interesting due to the neovasculogenesis and proliferation

• Macrophages present plasticity to change the behaviour to M1 or M2 and this property have a great potential in the Regenerative Medicine
Thank you

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