PLATELET RICH PLASMA (PRP)

Sev Hrywnak DPM,MD
Board Certified in Functional Medicine

International Peptide Society
Genetics in Medicine
Critical Care Medicine

Faculty, Department of Family Practice
Northwestern School of Medicine

Instructor, Scholl College of Podiatric Medicine
Internal Medicine, Physical Diagnosis, Immunology,
Practice Management, Business Law

Industrial Engineering College of Chicago
Instructor, Calculus for Physics

HISTORY OF PRP

1970 PRP Introduced

1987 Clinical Use in Cardiovascular Surgery

1990 128 Publications on PRP

1999 Companies discover PRP, commercialize PRP

2006 PRP used in sports medicine and pain management

2025 2,681 Publications to date on PRP, Many Medical specialties Use

WHAT IS A PLATELET

Cytoplasmic fragments of magakaryocytes formed in the bone marrow and circulation of the blood

Platelets contain small encapsulated structures called alpha-granules, dense granules, and lysosomes

Alpha-granules contain numbers of growth factors

Physiological Role of Platelets in Healing

Platelet Activation

When tissue injury occurs, platelets adhere to exposed collagen via glycoprotein receptors (GP Ia/IIa and GP VI).

They become activated and release the contents of their **α-granules**, **dense granules**, and **lysosomes**, initiating the **hemostatic and healing cascade**.

Granule Contents

- **Alpha granules:** Contain growth factors, cytokines, and adhesion proteins.
- **Dense granules:** Contain ADP, serotonin, calcium, and catecholamines, which amplify platelet activation.
- **Lysosomes:** Contain enzymes that remodel the extracellular matrix.

Physiological Phases of PRP in Healing

Inflammation phase: Growth factors recruit macrophages and fibroblasts.

Proliferation phase: Stimulates angiogenesis and fibroblast proliferation.

Remodeling phase: Promotes collagen deposition and tissue maturation.

Molecular Components and Their Roles

Major Growth Factors Released:

These growth factors bind to **specific receptors on target cell membranes**, triggering **intracellular signaling cascades** that activate gene transcription and protein synthesis.

Growth Factor	Source	Function
PDGF (Platelet-Derived Growth Factor)	α-granules	Stimulates fibroblast and smooth muscle proliferation; chemotactic for macrophages.
TGF-β (Transforming Growth Factor-β)	α-granules	Promotes collagen synthesis, inhibits inflammation.
VEGF (Vascular Endothelial Growth Factor)	α-granules	Stimulates angiogenesis and endothelial cell migration.
EGF (Epidermal Growth Factor)	α-granules	Stimulates epithelial and fibroblast proliferation.
IGF-1 (Insulin-like Growth Factor-1)	Plasma/α-granules	Promotes cell growth and differentiation.
FGF (Fibroblast Growth Factor)	α-granules	Stimulates angiogenesis and fibroblast proliferation.

Molecular Biology and Signal Transduction

Receptor Binding

Most PRP growth factors act via **receptor tyrosine kinases (RTKs)**.
When a growth factor binds, it causes receptor **dimerization and autophosphorylation**, which initiates downstream signaling.

Key Signaling Pathways

MAPK/ERK Pathway:
Promotes **cell proliferation and differentiation**.
PI3K/Akt Pathway:
Enhances **cell survival, angiogenesis**, and **metabolism**.
JAK/STAT Pathway:
Regulates **cell growth and immune modulation**.
SMAD Pathway (for TGF-β):
Controls **collagen synthesis** and **fibrosis regulation**.

Gene Expression Outcomes

These cascades result in transcription of genes involved in:

- Extracellular matrix (ECM) synthesis
- Cell cycle progression
- Anti-apoptotic proteins
- Cytoskeletal remodeling

PRP–Stem Cell Interaction

PRP growth factors stimulate **mesenchymal stem cell (MSC)** proliferation and differentiation. Key interactions include:

- **PDGF and TGF-β:** Promote MSC migration and differentiation into fibroblasts and osteoblasts.
- **VEGF:** Enhances endothelial progenitor cell recruitment for neovascularization.
- **IGF-1:** Supports chondrogenic and myogenic differentiation.

This regenerative influence explains PRP's application in **orthopedics, dentistry, and dermatology**.

Fibrin Matrix Biology

When activated (often with calcium chloride or thrombin), PRP forms a **fibrin scaffold** that acts as:

- A **mechanical matrix** for cell migration.
- A **slow-release reservoir** for growth factors.
- A **hemostatic plug** aiding initial wound closure.

The fibrin mesh is gradually degraded by **plasmin**, allowing controlled release of bioactive molecules over several days.

Cellular Targets and Regenerative Effects

PRP’s effects on different tissues are mediated by activation of:

- **Fibroblasts** → collagen and ECM production
- **Endothelial cells** → angiogenesis
- **Chondrocytes and osteoblasts** → cartilage and bone regeneration
- **Keratinocytes** → epithelialization

Physiological Outcomes

The coordinated effects include:

- Enhanced **tissue perfusion and vascularization**
- Accelerated **cell proliferation and migration**
- Reduced **inflammation**
- Improved **collagen architecture**
- Increased **mechanical strength** of healed tissue

Summary Diagram

Injury → Platelet activation → Growth factor release → Receptor activation → Signal transduction (MAPK, PI3K/Akt) → Gene expression → Cell proliferation, ECM remodeling, angiogenesis → Tissue repair

Pure Platelet-Rich Plasma (P-PRP)

Composition

- High concentration of **platelets**
- **Low or absent leukocytes (WBCs)**
- **Liquid plasma** with a **low-density fibrin network**

Preparation

- Usually produced by **single centrifugation**, separating platelets from red and white cells.
- The upper plasma layer containing platelets is collected, minimizing buffy coat contamination.

Pure Platelet-Rich Plasma (P-PRP)

Mechanism

- Upon activation (with calcium chloride or thrombin), it releases growth factors **quickly**, leading to a **short-term but potent regenerative signal**.

Applications

- **Aesthetic medicine** (e.g., facial rejuvenation, hair restoration)
- **Dermatology**
- **Ophthalmology** (e.g., corneal healing)
- **Tendon and soft-tissue repair** where minimal inflammation is desired

Advantages

- Minimal inflammatory reaction
- Easy to inject (remains liquid)
- Useful for delicate tissues

Leukocyte- and Platelet-Rich Plasma (L-PRP)

Composition

- High concentration of **platelets**
- **High content of leukocytes**
- **Liquid plasma** with **minimal fibrin mesh**

Preparation

- Usually prepared using a **double-spin method**, capturing both the buffy coat (rich in WBCs) and platelets.

Mechanism

- Upon activation, platelets release growth factors while leukocytes release **cytokines** that modulate inflammation and immunity.

Leukocyte- and Platelet-Rich Plasma (L-PRP)

Applications

- **Orthopedics and sports medicine** (tendon injuries, osteoarthritis)
- **Wound healing** and **chronic ulcers**
- **Dental and maxillofacial surgery**

Advantages

- Enhanced **antimicrobial** and **immune-regulatory** effects
- Stronger initial **inflammatory stimulation** that promotes tissue regeneration

Considerations

- May cause **more post-injection inflammation or pain** due to leukocyte content

Pure Platelet-Rich Fibrin (P-PRF)

Composition

- Platelet concentrate within a **fibrin clot or gel**
- **Low leukocyte** content
- **Dense fibrin matrix**

Preparation

- No anticoagulant is used.
- Blood is centrifuged once, allowing natural coagulation to form a **fibrin clot** containing trapped platelets.

Pure Platelet-Rich Fibrin (P-PRF)

Mechanism

- Platelets embedded in the fibrin network **slowly release growth factors** over several days, providing **sustained tissue stimulation**.

Applications

- **Dental and oral surgery** (socket preservation, sinus lift)
- **Plastic surgery**
- **Chronic wound healing**

Advantages

- Acts as a **natural scaffold** for cell migration and angiogenesis
- Long-term release of bioactive molecules

Leukocyte- and Platelet-Rich Fibrin -PRF)

Composition

- **High platelet** and **high leukocyte** content
- **Dense fibrin matrix**
- Forms a **solid membrane or plug**

Preparation

- Natural coagulation (no anticoagulant)
- Centrifugation at specific speeds allows trapping of both platelets and leukocytes within the fibrin clot.

Mechanism

- Provides a **three-dimensional fibrin scaffold** with platelets and leukocytes releasing growth factors over **7–10 days**.
- Leukocytes also contribute to **angiogenesis and immune regulation**.

Leukocyte- and Platelet-Rich Fibrin (L-PRF)

Applications

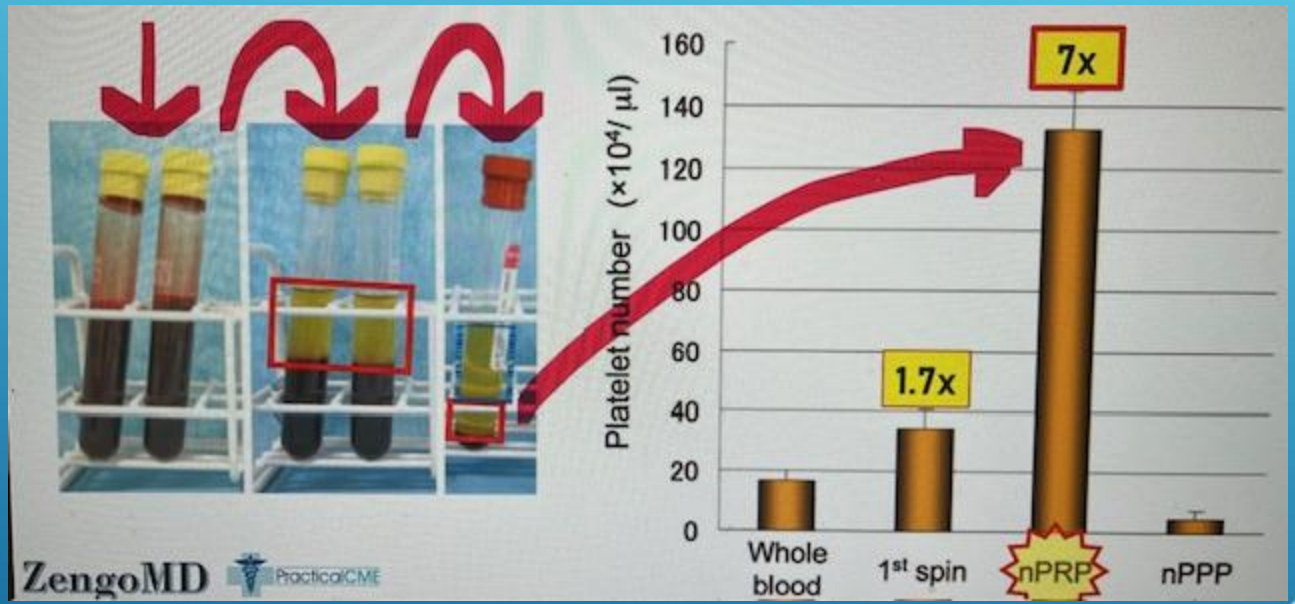
- **Bone grafting and dental implant surgery**
- **Chronic wounds and ulcers**
- **Reconstructive surgery**

Advantages

- Sustained release of growth factors
- Promotes both **regeneration and infection control**

Comparative Summary

Type	Platelets	Leukocytes	Fibrin Density	State	Release Duration	Common Use
P-PRP	High	Low	Low	Liquid	Fast (hours)	Aesthetics, dermatology
L-PRP	High	High	Low	Liquid	Fast (hours)	Orthopedics, wounds
P-PRF	High	Low	High	Gel	Slow (days)	Dental, plastic surgery
L-PRF	High	High	High	Solid membrane	Sustained (days–weeks)	Oral, bone, chronic wounds


Platelet number (×10⁴/ μl)
160
140
120
100
80
60
40
20
0
7x
1.7x
Whole blood
1st spin
nPRP
nPPP
ZengoMD
PracticalCME

What “Activation” Means

Activation refers to the process of stimulating platelets in PRP to **degranulate** — that is, to release the bioactive molecules stored in their **α-granules** and **dense granules**.
This causes platelets to:

- Change shape (from discoid to spiny)
- Aggregate
- Release **growth factors**, **cytokines**, and **adhesion molecules**
- Initiate **fibrin polymerization** (if fibrinogen is present)

The goal of activation is to make these growth factors **available to tissues** at the right time and rate for optimal healing.

When to Activate PRP

Exogenous Activation (Before Injection)

PRP is activated *outside the body* immediately before injection or application.
This is typically done when:

- A **gel or fibrin matrix** is desired (for wound coverage, grafts, or surgical sites)
- The target tissue has **low endogenous activation potential** (e.g., avascular structures)
- The clinician wants **immediate release of growth factors**

Used in:

- **Dental and oral surgery** (membrane or graft coating)
- **Plastic surgery and dermatology** (topical gel for wound healing)
- **Chronic ulcers**
- **Bone grafting**

When to Activate PRP

Endogenous Activation (In Vivo)

Here, PRP is injected in its **non-activated liquid form**.
Platelets are naturally activated by **exposure to tissue collagen, thrombin, and calcium ions** once injected.

Used in:

- **Intra-articular injections** (e.g., knee osteoarthritis)
- **Tendinopathies**
- **Muscle or ligament injuries**
- **Hair restoration**

This allows for **slower, sustained release** of growth factors directly at the injury site, mimicking physiological healing.

Agents Used to Activate PRP

Activator	Mechanism	Effect	Common Use
Calcium chloride ($CaCl_2$)	Provides Ca^{2+} ions that trigger the clotting cascade and fibrin polymerization	Forms a **soft gel**; moderate release speed	General PRP activation; topical or injectable forms
Calcium gluconate	Similar to $CaCl_2$ but milder and slower	Gradual activation	Intra-articular and dermatologic PRP
Autologous thrombin	Directly converts fibrinogen → fibrin and activates platelets via PAR receptors	Rapid clot formation; strong activation	Surgical or dental PRP gels
Bovine thrombin	Historically used but may cause immune reactions	Rapid, strong activation	Rarely used now due to antibody risk
Collagen exposure	Endogenous activator within tissue	Natural activation	Occurs *in vivo* after injection

Physiologic Mechanism of Activation

When activated (by any of the above):

- **Thrombin** converts **fibrinogen** (from plasma) into **fibrin monomers**, forming a clot or scaffold.
- **Platelets bind fibrin**, undergo **shape change**, and release:
  - **PDGF** (Platelet-Derived Growth Factor)
  - **TGF-β** (Transforming Growth Factor Beta)
  - **VEGF** (Vascular Endothelial Growth Factor)
  - **EGF**, **IGF-1**, and others
- These growth factors then bind to receptors on local cells, initiating **tissue regeneration and angiogenesis**.

Physiologic Mechanism of Activation

The **speed and duration of release** depend on how the PRP was activated:

- **Strong activators (e.g., thrombin)** → rapid burst release (within minutes)
- **Mild activators (e.g., $CaCl_2$ or natural exposure)** → slower, prolonged release (over hours to days)

Clinical Decision Summary

Objective	Activation Method	Timing
Immediate release & clot formation	Thrombin or $CaCl_2$	Before injection
Gradual release & deeper diffusion	Endogenous (no pre-activation)	After injection
Scaffold or membrane creation	Thrombin + Ca^{2+} (double activation)	Before topical use

Important Considerations

- **Do not over-activate**: premature clotting can trap growth factors, reducing their bioavailability.
- **Use autologous activators when possible** to minimize immune response.
- **Ensure timing**: Activated PRP must be used promptly (within minutes), as it begins to gel and lose flowability.
- **Tissue type matters**: avascular tissues (like cartilage) may benefit from mild pre-activation, while vascular tissues activate PRP naturally.

Preparation Principles

PRP is derived from **autologous blood**, typically drawn from the patient's arm (10–60 mL depending on the intended use).
The objective is to **concentrate platelets and growth factors** while minimizing contamination with red and white blood cells.

Basic steps

1. **Blood collection** – Whole blood is drawn into tubes containing an **anticoagulant** (usually ACD-A or sodium citrate).
2. **Centrifugation** – The blood is spun in a centrifuge to separate its components based on density.
3. **Fraction isolation** – The **plasma layer rich in platelets** is carefully removed.
4. **Activation (optional)** – Calcium chloride or autologous thrombin may be added to trigger platelet degranulation before or after injection, depending on the protocol.

Centrifugation Concept

The centrifugation process separates:

- **Red blood cells (bottom layer)**
- **Buffy coat (white blood cells + platelets)**
- **Plasma (top layer)**

There are **single-spin** and **double-spin** systems:

- A *single spin* yields **platelet-poor plasma (PPP)** and **platelet-rich plasma (PRP)** in one step.
- A *double spin* performs an initial separation and then a second, faster spin to further concentrate platelets.

Volume and Concentration Concepts

- The **final PRP volume** typically represents **10–20 % of the initial blood volume** after concentration.
- The **platelet concentration** can range from **3× to 8× baseline**, depending on method and spin parameters.
- The goal is a balance between **growth-factor richness** and **plasma usability** (too concentrated or leukocyte-heavy PRP may increase inflammation).

Clinical Administration Principles

Injection technique, target joint, and dosing frequency depend on:

- **Indication** (e.g., osteoarthritis, tendinopathy, wound healing)
- **Joint size and anatomy**
- **Degree of pathology and physician protocol**

In general, clinicians tailor:

- **Volume** – proportional to the target tissue capacity and diffusion area
- **Frequency** – often delivered as part of a multi-session protocol to achieve cumulative biological stimulation
  Only a qualified physician can determine these safely.

Biologic Rationale

The therapeutic effect arises when **activated platelets release growth factors** such as PDGF, TGF-β, VEGF, and IGF-1.
These factors stimulate:

- **Fibroblast and chondrocyte proliferation**
- **Extracellular-matrix synthesis**
- **Angiogenesis and anti-inflammatory modulation**

Proper preparation ensures platelets remain intact until use, optimizing bioactivity at the injection site.

Key Safety Considerations

- Use **sterile, closed systems** to prevent contamination.
- Maintain **temperature control** to preserve platelet viability.
- Avoid hemolysis during pipetting.
- Always perform under **medical supervision** following approved protocols.

Summary

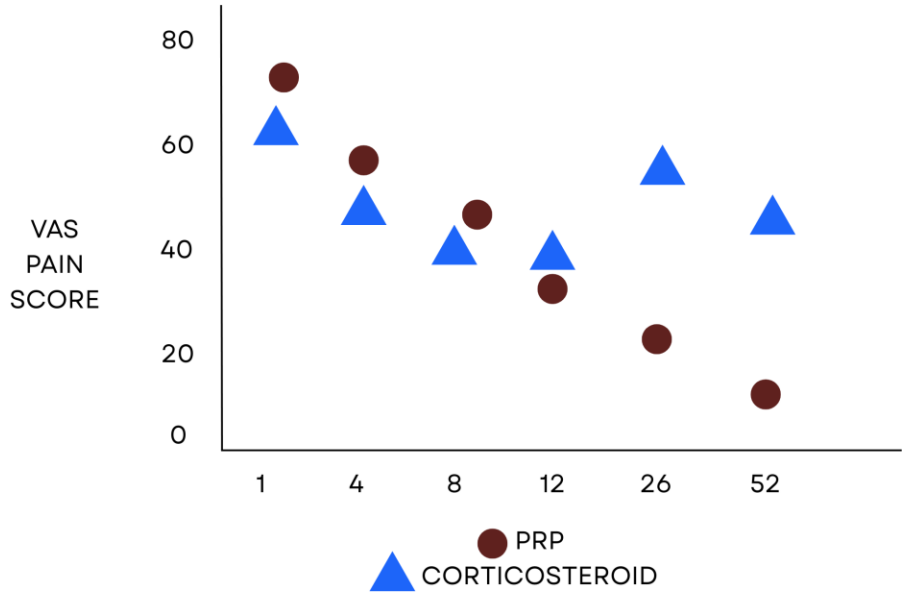
Step	Purpose	Controlled Variable
Blood draw	Obtain autologous sample	Volume, anticoagulant
Centrifugation	Separate components	Relative centrifugal force, time
Plasma extraction	Isolate platelets	Layer accuracy
Activation (optional)	Trigger growth-factor release	Calcium/thrombin
Injection (clinical)	Deliver regenerative stimulus	Volume, location, interval — **physician-determined**

IMPORTANT POINTS TO REMEMBER

1. No Marcaine ! Marciane destroys platelets. 1% lidocaine is ok
2. Stop NSAIDs 2 weeks prior to injection
3. No NSAID use up to 6 weeks post injection
4. Injections for the foot and ankle: 3 injections spaced 10-14 days apart.

PRP VS. CORTICOSTEROID

Ankle Tendonitis (AJSM 2020)



Thank you !