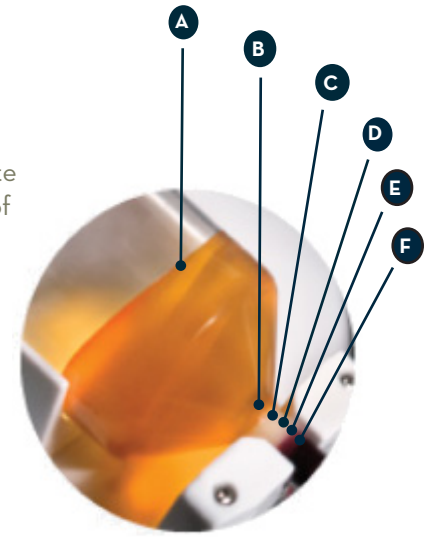
# Formulation Rationale

## Leukocyte Rich vs. Leukocyte Poor Biologics

This document provides an overview of the components in Platelet Rich Plasma (PRP) and their roles in healing.

### What are the Components of Platelet Rich Plasma (PRP)?

PRP is concentrated into six main layers (from lightest to heaviest): Plasma, Platelets, Monocytes, Lymphocytes, Neutrophils, and Erythrocytes. Neutrophils are considered granulocytes and there are also two other forms: Eosinophils & Basophils, but they are present in very low (sometimes undetectable) concentrations. Each layer overlaps in the density gradient and plays an important role in the healing process. Below is an outline of each layer and its role in healing:[1]




A. **Plasma** (1.025 – 1.029 g/ml): Transport nutrients, blood cells, provide clotting factors

B **Platelets** (1.060 – 1.067 g/ml): Store Growth Factors (GFs) & Cytokines, Regulate Hemostasis, and attract White Blood Cells (WBCs)

C. **Monocytes** (1.062 – 1.068 g/ml): Release Matrix Metalloproteinases (MMPs) to regulate the turnover of extracellular matrix (ECM) allowing for cellular migration and induction of healing. Transform into macrophages, secrete GFs to support neoangiogenesis, remove neutrophils

D. **Lymphocytes** (1.068 – 1.072 g/ml): Release factors to control inflammation & regulate cell growth

E. **Neutrophils** (1.080 – 1.090 g/ml): Migrate to damaged tissue, induce phagocytosis of debris, necrotic tissue and microbes. It is important to note that Neutrophils are the heaviest White Blood Cell (WBC)

F. **Erythrocytes** (1.086 – 1.100 g/ml): Increase activation & release of cytokines from platelets

### PRP & cBMA Role in Healing

WBCs play a large role in the healing cascade. They are in a resting (non-inflammatory) state until two activating signals are launched: Priming and Activation Signals.[2]

Hemostasis (Seconds to Hours) > Acute Inflammation (Hours to Days) > Proliferation (Days to Weeks) > Remodeling (Weeks to Months) > Healed Tissue

When PRP is injected, it begins to clot which signals Neutrophils to be in a primed (but not activated) state. In this state, they help platelets produce anti-inflammatory signals (such as IRAP – IL-1ra). This helps push the Wound Healing cascade forward instead of prolonging the Inflammation phase.[2]

### Defining Leukocyte Rich-PRP and Leukocyte Poor-PRP

Technically speaking, leukocyte poor (LP)-PRP is defined as a platelet concentrate in which all WBCs are depleted. However, based on the current clinical literature on this topic most clinicians are concerned with granulocyte (inflammatory WBC) content. Since neutrophils make up the majority of granulocytes, LP-PRP has rather become a moniker to describe a PRP preparation which is neutrophil-reduced.

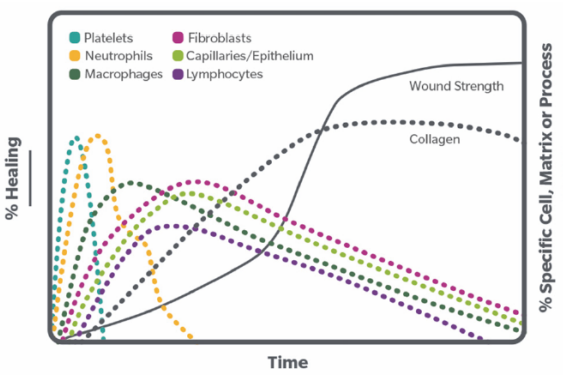



Figure 1: Role of different cell types. Note: the importance Neutrophils and Lymphocytes play in wound healing.[3]

Related to this, WBCs (including neutrophils) play a large role in the healing cascade (Figure 1)[3], thus WBCs should not be considered "undesirable" because one of the main functions of platelets is to recruit WBCs (including neutrophils) to a wound.[3] Please note that when you deplete WBCs you will also deplete platelets because of the overlapping density layers.

WBCs change function based on need. For example, if you have an infection, WBCs can be inflammatory (normally, they'd induce tissue repair). When using cBMA, MSCs are also anti-inflammatory, and cBMA contains concentrated IRAP.[4] Monocytes & Neutrophils also secrete IRAP.[5]

**To Summarize:**

- Depending on their environment, WBCs can hinder or induce healing.
- Platelets degranulate and release GFs/cytokines, which recruit WBCs to the wound site.
- Platelets recruit/attract WBCs to a wound site.
- Platelets and GF yields are decreased when WBCs are depleted. [3, 6, 7, 8, 9]

Red Blood Cells (RBCs) do have an effect on synoviocyte viability (important for joint injections), but the effect with Magellan® PRP/cBMA hematocrit (Hct%) even on standard cycle is very little compared to high Hct% systems such as Harvest, Biomet, Celling, & Emcyte.[10]

Though Magellan® is considered a Leukocyte Rich (LR) concentration device, Cassano 2016 shows that the standard cycle produces a Neutrophil-reduced concentrate (reduces neutrophils by ~0.48x baseline but concentrates helpful Monocytes by 11.6x baseline) (Figure 2).[4] The standard cycle also produces a high number of platelets (~8x baseline), MSCs (5.3x baseline CFU-fs), and HSCs (~5-6x baseline).[11]

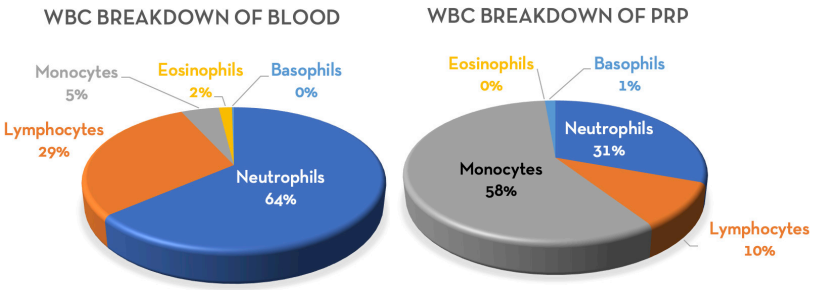



Figure 2: Breakdown of WBC Components Before & After Centrifugation with Magellan® System using standard settings.[4]

## Leukocyte Rich-PRP vs Leukocyte Poor-PRP

There are only two head-to-head studies of LP-PRP vs LR-PRP, which show no clinical difference between the two types of PRP (yet LR-PRP gave an initial pain/swelling response).[6, 12] There is no difference in adverse events between LP & LR.[7, 13]

## When to Choose PRP vs. cBMA

There is no literature which advises use of one over the other, but cBMA is generally used in more "difficult to treat" cases whereas PRP is used in lesser "difficult to treat" cases. cBMA has been reported in high-level literature to be clinically superior when compared to control groups in treating knee OA.[14, 15, 16] The PRP repair mechanism is unlikely to involve a direct mediation of chondrogenic repair,[17] rather helping to repair a physiologic issue (such as pH, inflammatory signaling, cellular signaling, etc), cBMA treatment may provide viable cells to repair the damaged tissue. It is best to choose PRP/cBMA treatment based on patient need(s).


**References**

**1.** Pavlovic, V et al (2016) Platelet Rich Plasma: a short overview of certain bioactive components. Open Med. 11: 242-247. **2.** Parrish, WR and Roides, B (2017) Physiology of Blood Components in Wound Healing: an Appreciation of Cellular Co-Operativity in Plateletu7 Rich Plasma Action. J Exerc Sports Orthop 4(2):1-14. **3.** King, W et al (2018) Role of White Blood Cells in Blood- and Bone Marrow-Based Autologous Therapies. BioMed Research International, Article ID 6510842. **4.** Cassano, J.M. et al. (2016) Bone marrow concentrate and platelet-rich plasma differ in cell distribution and interleukin 1 receptor antagonist protein concentration. Knee Surg. Sports Traumatol Arthrosc. 26, 333 - 342. **5.** Hernigou, P et al. (2016) Local transplantation of bone marrow concentrated granulocytes precursors can cure without antibiotics infected nonunion of polytraumatic patients in absence of bone defect. Int. Orthop. 40:2331–2338. **6.** Filardo, G et al (2012) Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc. 20: 2082-91. **7.** Riboh, JC et al (2016) Effect of leukocyte concentration on the efficacy of platelet-rich plasma in the treatment of knee osteoarthritis. Am J Sports Med. 44: 792-800. **8.** Fitzpatrick, J et al (2017) Analysis of Platelet-Rich Plasma Extraction: Variations in Platelet and Blood Components Between 4 Common Commercial Kits. Orthop J Sports Med. 5: 2325967116675272 **9.** Ziegler, CG et al. (2019) Characterization of Growth Factors, Cytokines, and Chemokines in Bone Marrow Concentrate and Platelet-Rich Plasma: A Prospective Analysis. Am J Sports Med. 47: 2174-2187. **10.** Braun, HJ et al (2014) The Effect of Platelet-Rich Plasma Formulations and Blood Products on Human Synoviocytes: Implications for Intra-articular Injury and Therapy. Am J Sports Med. 42: 1204-1210. **11.** AMSI Source: TS-001A-008 **12.** Hanisch, K & Wedderkopp, N (2019) Platelet-rich plasma (PRP) treatment of noninsertional Achilles tendinopathy in a two case series: no significant difference in effect between leukocyte-rich and leukocyte-poor PRP. Ortho Res & Rev 11: 55-60. **11.** Le ADK et al (2019) Platelet-rich plasma. Clin Sports Med. 38: 17-44. **12.** Upadhyay, S et al (2014) Randomized blinded comparative analysis of autologous mononuclear medullary fraction and viscosupplementation in grade 0 and grade 1 radiographic scale of knee osteoarthritis. Indian J Pain 28: 134-142. **15.** Goncars, V et al (2017) The comparison of knee osteoarthritis treatment with single-dose bone marrow-derived mononuclear cells vs. hyaluronic acid injections. Medicina 53: 101-108. **16.** Garay-Mendoza, D et al (2018) The effect of intra-articular injection of autologous bone marrow stem cells on pain and knee function in patients with osteoarthritis. Int J Rheum Dis. 21: 140-147. **17.** Liou, JJ et al (2018) Effect of Platelet-Rich Plasma on Chondrogenic Differentiation of Adipose- and Bone Marrow-Derived Mesenchymal Stem Cells. Tissue Engineering. 24: 1432-1443. .



45 South Street, Hopkinton, MA 01748