# WHY TROPOCELLS® IS CONSIDERDTHE BEST PRP SYSTEM IN THE MARKET

Platelet-rich plasma (PRP) is effective treatment for many orthopedic conditions. However, many studies using first-generation PRP systems have attempted to show PRP's effectiveness but have failed. The reason is that there is a lack of standardization of cellular content of PRP formulations and cellular ratios in the final treatment product. Experts explain that "not all PRP is created equal" and have only recently defined what makes PRP effective (TOBI 2018). The most recent formula of PRP defined by experts as the following features:

· Minimize red blood cells (RBCs)

· Minimize neutrophils

· Maximize monocytes

· Maximize lymphocytes

Here are studies backing what experts are defining as the optimal PRP formula:

**RBCs are catabolic (degrading and inhibitory):**

o RBCs cause apoptosis of native tissue, specifically chondrocytes (Hoovield 2003, Jansen 2007, Melchiorre 2017), synoviocytes (Sethi 2018), and chondrocyte progenitor cells (Roosendaal 1997)

o RBCs disable proteoglycan synthesis in joints (Castillo 2011, Amable 2013, Kawase 2015, Cassano 2018)

o RBCs impair the recruitment, migration, differentiation, and proliferation of mesenchymal stem cells (MSCs) (Sethi 2018), the cells responsible for native healing and PRP's effectiveness

o RBCs are considered to be not only contaminants in PRP, but antagonists to the entire healing process. Experts are calling for a standard where no more than 10% RBC contamination is allowed—this standard would remove over half of currently available PRP systems (Chahla 2018)

**Neutrophils are catabolic:**

o Neutrophils kill synoviocytes and chondrocytes (Braun 2014)

o Neutrophils degrade native collagen (Wright 2010) as well as the fibrin matrix that PRP creates in the target area (Sampson 2008)

· Neutrophils are pro-inflammatory. They cause platelets to selectively release pro-inflammatory markers that enable breakdown of tissue. An inflammatory flare is quite painful and can last for days.

o Neutrophils release pro-inflammatory cytokines directly and influence neighboring cells to do the same (Pifer 2014)

o The proteins released by neutrophils cause a huge flare after PRP injections (Filardo 2012)

**Monocytes are anabolic:**

o Monocytes stimulate collagen production in skin (Gonzales 1988), tendons (Murray 2009), gums (Nakao 1995), and cartilage (Murray 2009)

o Monocytes directly recruit MSCs and aid their differentiation and proliferation (Gebraad 2018)

o Monocytes and platelets have a synergistic anabolic effect when working together (Murray 2009, Yoshida 2013)

o Monocytes are primarily responsible for converting the inflammatory phase to the recovery phase of healing (Tuomi 2003, Tidball 2007, Harmon 2011)

o In an inflammatory environment, monocytes secrete pro-inflammatory interleukins, but contrary to neutrophils which secrete IL-1 (inflammatory and catabolic), monocytes secrete IL-6 which is inflammatory and anabolic (Naldini 2008)

o Monocytes are crucial for wound healing (Brancato 2011) and ligament strength (Yoshida 2013)

**Lymphocytes are anabolic** (Lana 2017, Yoshida 2013). They support monocytes in function and mechanism and help MSCs proliferate (Barbul 1989).

First generation PRP systems all produce end formulations which are highly contaminated with RBCs and neutrophils. When treating patients with first-generation PRP, providers must warn patients of an inflammatory flare and tell them to expect intense pain that may last for several days.

These older first-generation PRP systems use the buffy coat method for collecting PRP which is very imprecise. The illustrations below show a sample of blood after it has spun in a centrifuge. Because RBCs have the highest density, they end up being concentrated at the bottom of the sample. On top of the RBCs is a concentrated collection of platelets, monocytes, lymphocytes, and neutrophils, all of which comprise the buffy coat (represented as the blue layer in the diagram on the left). Buffy coat PRP systems require collection of the entire buffy coat in order to get a high concentration of platelets; during aspiration of the buffy coat contents, numerous RBCs inevitably end up in the sample.

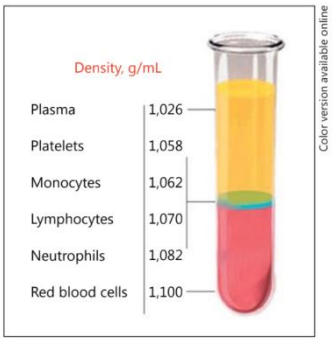


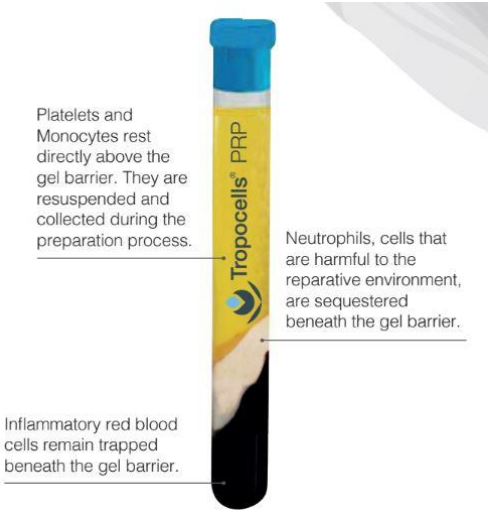

**Fig. 1.** After centrifugation, the blood components (red blood cells, leukocytes, and platelets) are separated from the plasma due to their different densities. The platelets have the lowest density. Adapted from Dohan Ehrenfest et al. [38].

Because there is no barrier between the levels during aspiration of the buffy coat into the syringe, numerous RBCs and neutrophils will contaminate the PRP specimen.

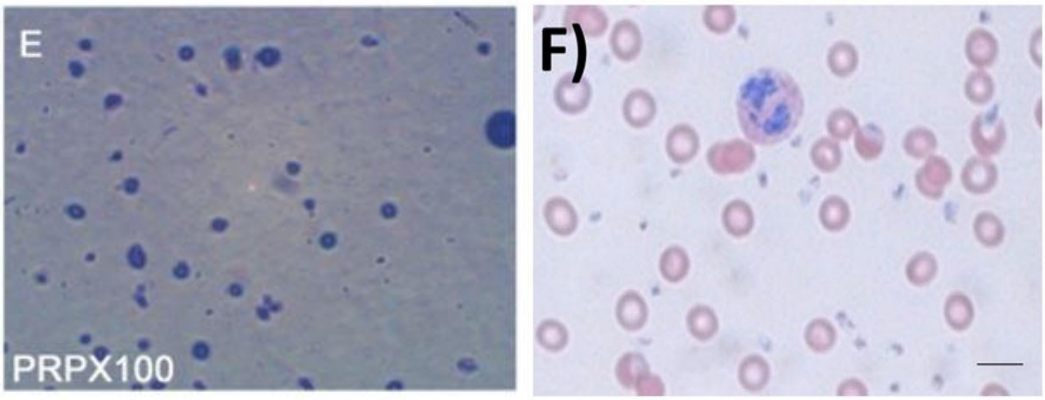
**The reason Tropocells PRP is the next generation preparation system is that it is able to consistently sequester the RBCs and neutrophils beneath a physical gel barrier while preserving monocytes, lymphocytes, and platelets above the gel barrier**. The innovative design is in a gel which has an ideal density to separate the anabolic monocytes and lymphocytes from the undesirable RBCs and neutrophils and include them in the PRP. Here are cell counts in Tropocells PRP (Theodor 2009):

- RBCs (M/uL)
- 0.2 WBCs (K/uL)
- 8.5% Neutrophils
- 86.2% Mononuclear cells (lymphocytes and monocytes)

The diagram below illustrates how the gel barrier can preserve the monocytes, lymphocytes, and platelets above it and sequester the pro-inflammatory and catabolic neutrophils and RBCs beneath it.



Below are microscopic images of smears in which you can see the difference in the formulation of Tropocells PRP (E) and Harvest PRP which uses first generation buffy coat technology (F). The purple specks are platelets. You can see that the number of platelets is relatively equal. The pink donuts are RBCs and are found only in picture F, the buffy coat method of PRP preparation. The larger cells are WBCs; in the Tropocells PRP (E) it is a monocyte which is anabolic; in the Harvest PRP buffy coat sample (F), the WBC is a neutrophil which is catabolic.




**Because of innovative gel-barrier separation technology, Tropocells PRP can reliably deliver the optimal PRP formula of almost 100% removal of RBCs, 93% removal of neutrophils, and preserving the monocytes and lymphocytes. This is an innovative and technological breakthrough that has mad Tropocells PRP the new standard.**

Lack of RBCs and neutrophils means no painful inflammatory flare to patients. It also means a potential for better clinical outcomes. In our clinical experience, it is so gratifying to avoid the inflammatory flare that caused patients to limp around in pain for days. Since adopting the Tropocells PRP system, our patients are enjoying a relatively painless procedure and are experiencing more positive and longer-lasting outcomes.

It should also be noted that Tropocells has other distinct advantages over other PRP systems that indirectly improve patient outcomes. These are discussed in detail in subsequent sections:

- Buffered anticoagulant
  - Higher platelet yield
  - Less pain during injection
- Specially-treated collection tubes
  - Higher platelet yield
  - Less risk of premature platelet activation
- Simple preparation process within a closed system (PRP is generated in the same blood collection tube)
  - Higher platelet yield
  - Less risk of bacterial contamination
  - Less risk of needle-stick injury

In summary, PRP is a useful treatment for multiple orthopedic applications, many of which have very limited effective treatment options. Because Tropocells PRP is now available with its next-generation, innovative gel barrier technology, clinical treatment for many various conditions is likely to improve.

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- **Buffered anticoagulant**
    - Higher platelet yield
    - Less pain during injection
- **Specially-treated collection tubes**
    - Higher platelet yield
    - Less risk of premature platelet activation
- **Tropocells is a closed system and has a very simple preparation process** (PRP is generated in the same blood collection tube)
    - Higher platelet yield
    - Less risk of bacterial contamination
    - Less risk of needle-stick injury

**Safety Considerations**

Historically, PRP is a treatment that is considered safe. Because the PRP sample originates from a patient's own body, the risk of an allergic reaction is vanishingly rare. The only additive is the anticoagulant in the sample and this is generally well-tolerated. The inherent risks with any PRP system are similar across the board—transmission of a blood-borne illness, infection, and pain at the injection-site.

That being said, **the Tropocells PRP system has some distinct advantages over other PRP systems**.

- **Entirely closed system—decreased risk of external contamination**
- **Minimal transfers—decreased risk of external contamination**
- **Less blood required per treatment site**
- **Buffered anticoagulant—less pain during injection**

Although not a guarantee, a fully-closed system minimizes the risk of infectious contamination. Many PRP systems require a step when the blood is exposed to air. Since PRP systems are usually at the point of care and not under a hood, the risk of contamination is increased.

Similarly, each time the blood has to change containers carries a small but measurable risk. The "next-generation" Tropocells PRP system has developed **a simplified preparation process** in which the blood into the same container that undergoes centrifugation. The one and only transfer is to draw up the PRP into the injection syringe. Other PRP systems require multiple transfers.

Because of minimal transfers and proprietary treatment of the vacutainers (to avoid premature clumping or activation), **the amount of blood drawn from a patient is about 3x less than what other PRP systems require**. Less blood required decreases the risk of blood borne illness, needle-stick injury, and vagal/near syncopal episodes.

Most PRP systems use ACD-A as their anticoagulant. This has a pH of 4.9 (Dhurat 2014). This acidic solution affects the PRP in numerous ways:

- The final PRP solution has a pH ranging from 6.5 to 7.1(Fitzpatrick 2017).
- Platelets have the tendency to clump and prematurely activate in an acidic solution (Callan 2009, Guder 2009)
- Acidic solutions are much more painful to inject than buffered solutions (Fukaya 2014)

Tropocells PRP includes a buffer to the ACD-A to maintain a physiologic pH during processing and injection—this results in **higher platelet yields and less pain during injection**.

**Simplicity**

The manufacturers of the Tropocells® PRP collection system have designed a system that is easy to understand and easy to use. Some of these factors seem inconsequential, but when performing PRP routinely a provider appreciates the attention to detail that makes the procedure run smoothly.

- Vacutainer instead of a syringe
    - Higher platelet yield because of treated tubes
    - Optimal formulation discards the inhibitory factors so the same clinical effect can be achieved with less blood volume
- Less blood drawn per treatment site
- Anticoagulant preloaded within the collection tube
- 10-minute single spin
- Minimal transfers—negligible time to process the PRP
- Very easy to learn and perform with negligible user-dependent variability

Most PRP systems provide a large syringe (30-60mL) for blood collection—a syringe so large that it requires two hands to withdraw the blood.

**Patients would rather give less than give more. The less the amount, the less anxiety a patient feels.** This also plays a factor in how long the blood draw takes: less blood equals less time. Less blood required also decreases the risk of blood borne illness, needle-stick injury, and vagal/near-syncopal episodes.

**How does Tropocells get by with less? It provides more efficient PRP. Almost 80% of the platelets in the blood sample make it into the final PRP product, while the platelet yield from buffy coat systems range from 13% to 72%.**

- The average buffy coat blood draw is 30mL per treatment area
- Because Tropocells is very efficient, it can produce the same dose of platelets (with less inhibitory contaminants) using far less blood (11mL)
- Efficiency is maximized by:
    - Minimizing transfers
    - Physiologic pH
    - Special treatment of the vacutainers' inner lining to remove silica particles and other residue that cause clumping and premature activation

**The Tropocells PRP collection tube is ready-to-go, pre-loaded with the correct amount of anticoagulant. Many PRP kits do not include anticoagulant and require the provider to acquire it beforehand. The phlebotomist must draw up the anticoagulant into the syringe before the blood draw. This is an extra step that takes time and involves a needle puncture—increasing the risk of contamination and needle-stick injuries.**

**The longer the spin time, the longer the patient is occupying valuable time and space. Some systems require two separate spins with manual processing steps in between, doubling the chance of delay, prolonging the spin time, and significantly increasing overall preparation time.**

**Minimizing transfers is in the best interest of both patients and providers. Each transfer requires additional time and increases the chance of human error. Additionally, each transfer also increases the risk of premature clumping or activation of platelets, infection, and needle-stick injury.**

**After watching a simple 4 minute instructional video, novice users can prepare Tropocells PRP as flawlessly as an experienced user. The preparation process is intuitive, simple, and easy to follow. The time it takes from patient arrival to injection time is 15 minutes.**

- **Ease of Preparation –**
    - Very little training or expertise is needed to prepare a high-quality PRP with Tropocells
    - Medical assistants are able to consistently prepare the Tropocells PRP in a closed, sterile system
- **Increased Efficiency --**
    - Total Preparation time is 15 minutes (10 minutes of that is centrifugation)
    - This system requires less time than other PRP systems (no transfers or 2$^{nd}$ spin is required)

    - Saves provider time
- **Low Initial Equipment costs**
    - Several PRP systems charge $8,000 - $15,000 for the centrifuge and processing equipment
    - The Tropocells centrifuge is much more affordable at $1200

- Due to the absence of RBCs and Neutrophils in Tropocells PRP, there is a lack of the inflammatory flare that is common following treatment with other PRP systems
- The inflammatory flare produced by other PRP systems often leads to increased costs resulting from additional office visits or by seeking other treatments such as acupuncture or physical therapy
- Providers using other systems frequently prescribe a few days of pain medications to manage the post-injection inflammatory response; there is no need for opiate pain medication(s) following PRP treatment with Tropocells due to lack of the inflammatory flare
- Another benefit of decreased pain following treatment is increased mobility which often leads to increased activity levels, weight loss, and improved cardiovascular health
- Since PRP is a newer treatment, no cost comparison between PRP systems has yet been published
- Due to the absence of RBCs and Neutrophils in Tropocells PRP, there is a lack of the inflammatory flare that is common following treatment with other PRP systems
- The inflammatory flare produced by other PRP products often leads to significant pain that often results in a few days of missed work
- There is no little to no downtime following treatment with Tropocells PRP – most patients are able to return to work the same day
- Since PRP is a newer treatment, no cost savings analysis between PRP systems has yet been published