CAREPRP SYSTEM EVALUATION IN VITRO TESTS & MEASUREMENTS

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Purpose

Perform a battery of tests and measurements on commercially available PRP systems, including the CARE platelet-rich plasma (PRP) system under evaluation to determine optimal performance criteria & competitive characteristics for each system under standard operating conditions. Identify system with highest overall performance characteristics applicable to CAREstream-associated therapeutic arenas.

Blood Collection

For each donor, 46cc - 115cc of whole blood was collected at a clinic nearby (Dr. Charles Gatt MD, University Orthopedic Associates). All sample analysis was conducted at the Princeton Innovative Center BioLabs (PICB) facility under physician supervision, according to a Blood Sampling Protocol (separate document). Anticoagulated blood samples were immediately transported to PICB following collection and processed using the various commercially available PRP systems, including CAREprp. PRP processing was completed within 1 hour of blood collection. One aliquot of unprocessed anticoagulated whole blood was retained, analyzed and served as a baseline for PRP hematology measurements.

Basic Tests

All tests were done at the PICB facility. Basic tests conducted on PRP, corresponding to FDA guidelines, consisted of:

1. Complete blood cell counts (CBC with differential - % & # of RBCs, granulocytes, monocytes, lymphocytes, platelets, total WBCs, etc.)
2. Complete platelet counts
3. Platelet recovery (% of total based on WB)
4. Platelet concentration factor
5. RBC content of the PRP

For the purposes of this initial study focusing on the platelet yield and RBC content, the Monocyte-Rich (MR) single-spin protocol was used throughout.

Materials and Methods

PRP Preparation

All samples collected were processed using the CAREprp system at the PICB Laboratory within 1 hour of blood draw. The samples were processed for the single-spin MR mode according to the standard product IFU.

Complete Blood Cell Counting with Differential (Hematology)

Complete blood cell counts were performed on the whole blood and the concentrated PRP using a Horiba ABX Micros60 hematology analyzer. Baseline whole blood measurements were performed on 500 uL sample of anticoagulated blood taken from each donor prior to PRP production. In a similar manner, aliquots of the final PRP were analyzed for each donor. All samples were run either in duplicate (whole blood) or triplicate (PRP).

Results (5/27/2022)

Table 1

DONOR	Volume (mL)	Platelet Count # x $10^3/mm^3$	Platelet Yield (%)	RBCs in PRP (# x $10^6/mm^3$)
CS				
Whole Blood (WB)	23.0	X^{avg} = 259	72.2	>0.01
PRP	13.0	331		0.07%
MW				
WB	23.0	153	85.7	≥0.01
PRP	13.0	232		0.12%
SO				
WB	23.0	135	82.3	>0.02
PRP	13.0	196		0.24%
RG				
WB	23.0	155	56.5	>0.01
PRP	13.0	155		0.06%

Summary

Plate Recovery (PR, % platelet yield), the fraction of total platelets recovered per volume of harvested anti-coagulated blood in the PRP, is given by the equation:

PR = (PRP volume) × (platelet count in PRP) / (WB volume) × (plate count in WB) *** 100%**

Where WB = whole blood

From the current study,

PR = 80.07% ± 4.6% (S.D.) N=4

Red blood cell (RBC) contamination of the PRP.

From the data shown in Table 1, far right column, the RBC content of the PRP produced by the CAREprp system *is at, or below, the limit of detection of the hematology analyzer.*

Comparison with other Commercially Available Systems

Platelet Recovery

Previous studies performed at our lab measured Platelet Recovery and RBC contamination in several other commercial PRP systems.

Run: 9/05/2019; Published: Appl. Sci. 2022; 12, 4965

Harvest Smart PReP 2 N=3

PR = 79.8% ± 12.9%

Eclipse PRP N=3

PR = 47.9% ± 34.8%

EmCyte Pure PRP N=4

PR = 68.2% ± 9.3%

Run: 9/10/2020

EmCyte Pure PRP N=2

PR = 53.9% ± 13.6&

RBCs in PRP = 0.12 ± 0.06 x $10^6/mm^3$

TropoCell (Estar) Large N=2
PR = 61.3% ± 6.7%
RBCs in PRP = 0.025 x10^6/mm^3

Run: 9/18/2020

TropoCell Small N=4
PR = 72.8% ± 18.7%
RBCs in PRP = 0.02 x 10^6/mm^3

Sanwell Large N=2
PR = 45.6% ± 19.8
RBCs in PRP = 0.013 x 10^6/mm^3

RBC Contamination

Comparing the results of this study to recent data published on other commercially available PRP systems demonstrates that the CAREprp system's performance is quite competitive. As mentioned above, the RBC content of the PRP produced by the CAREprp system *is at, or below, the limit of detection of the hematology analyzer.*

In a recent publication by Neculaes and co-workers (Appl. Sci. 2022, 12, 4965), the hematologic performance of several systems were compared in Table 2, shown below.

Table 2

	Average	Concentration Ratio (versus whole blood)
Whole Blood RBC ($\times10^{12}$ L^{-1})	4.06±0.78	
Harvest RBC ($\times10^{12}$ L^{-1})	0.86±0.41	0.21±0.09
Eclipse RBC ($\times10^{12}$ L^{-1})	0.05±0.06	0.01±0.02
EmCyte RBC ($\times10^{12}$ L^{-1})	0.20±0.09	0.05±0.02

Composition of whole blood, Harvest Smart PReP 2 PRP, Eclipse PRP and EmCyte PRP composition averaged for all four donors with uncertainty determined using standard deviation (S.D.).
WBC = white blood cells; RBC = red blood cells; PLT = platelets

Discussion

Platelet Yield and Adequate Platelet Concentration

- ***Platelet Yield/Recovery***
  Platelet yield is one of the least controversial parameters associated with PRP performance. Platelet yield, the fraction of platelets captured from a volume of whole blood, is a measure of a PRP system's efficiency. Less efficient systems require the harvesting of more blood to achieve a therapeutic platelet dose. Early PRP system were very inefficient some achieving as little as 17.6% yield (Weibrich et al 2002). Current systems are considerably more efficient. This means that a lower volume of whole blood drawn will be needed to produce an adequate dose/concentration of PRP.

From the data presented above, as well as data from other commercially available systems both published publicly or tested in our independent lab, it can be concluded that the CAREprp PRP System is capable of high platelet recovery/yield (>80%).

In contrast, the other systems demonstrated platelet recoveries/yields ranging from 79.8% (Harvest) down to 45.6% (Sanwell).

- ***Platelet Concentration/Platelet Dose***

  In contrast to platelet recovery, or even red blood cell contamination discussed below, optimal platelet concentration is a subject of pro-longed and heated debate among clinicians and academic researchers alike. Does the PRP have too few platelets or does it have too many platelets in the PRP preparation? There is ample evidence that for many clinical applications it is possible to have too low a platelet concentration. However, determining how low is too low can be difficult. Haynesworth et al. 2002 showed that in looking at directed stem cell migration a platelet concentration of no less than 1,000,000 platelet per microliter(uL) was needed to see MSC differentiation and proliferation as well as migration. Other has said that 300,000/uL is 'inadequate' for healing and tissue regeneration (Anitua et al. 2004). Robert Marx captured this idea in his 2001 paper entitled – "Platelet-Rich Plasma (PRP): What is PRP and What is not PRP?" (Marx RE. Implant Dent. 2001;10(4):225-8). Marx in 2004 wrote a PRP review article that summarized the debate up to that point. The review was well written and was so complete that future articles cite this article more than any other (over 1700 citations, according to Google Scholar). In the article, Marx denounced some PRP collecting systems saying that they were incapable of concentrating platelets high enough to create a clinical effect. He called for a standard—therapeutic PRP should be a minimum of 6ml with a minimum platelet concentration of 1million/uL—otherwise it should not be called PRP (Marx 2004).

  It is at this point that a distinction must be made between concentration factor, the relative increase in platelet concentration over the starting platelet concentration in the input whole blood (i.e., 2x, 4x, 6x, etc.) and the actual final platelet concentration in the PRP preparation. This last parameter has been called the 'platelet dose.' The platelet dose is a more accurate determination of PRP in that the normal platelet concentration range for adult humans is 150,000 to 400,000 platelets/uL. For a patient with less than 150,000 pts/uL (i.e., thrombocytopenic) more blood would have to be harvested because, even if the PRP system could produce a 4-5x concentration over the whole blood, the final PRP platelet concentration would fall significantly below 1 million platelets per uL.

Looking at the CAREprp system:

If….

We take the average to be 300,000 platelets/uL whole blood and,

we assume a platelet yield of 80%, the bottom 3.0 mL of PRP in a single-spin PRP run using the CAREprp in MR mode = 60% of the total platelet harvest of the CAREprp.

Then….

300,000 x 1000 x 23.0mL = 6.9 e^9 total platelets harvested

6.9 e^9 x 0.8 = 5.52 e^9 total platelets recovered

5.52 e^9 x 0.6 = 3.32 e^9 in the bottom 3.0mL

3.32 e^9/3 = 1.16 e^9 or 1.16 x 10^6/uL

Or somewhat higher concentration or platelet dose than that specified by Dr. Marx.

Red Blood Cells (RBCs) in PRP

While there are some researchers who consider red cell contamination of PRP to be beneficial (Parrish et al 2016), most authorities agree that RBCs should be avoided in the PRP production process. Some clinicians will say that RBCs should be minimized in PRP, however, as long as the other vital components of PRP are present (high intact platelet concentration, WBCs, plasma, fibrin, etc.) RBC s will not be detrimental. Other clinicians will say that RBCs in the PRP can cause pain or promote scar formation on injection. In contrast, the PRP literature has some very specific things to say about RBCs in PRP.

RBC contamination of PRP has been shown to have a detrimental effect on:

- **Fibrin Formation**
  Brezniak and co-workers demonstrated that free hemoglobin (such as produced in PRP through hemolysis) will inhibit fibrin polymerization and clotting. (Brezniak et al 1994).

- **Chondrocytes and Bone Formation**
  Chondrocytes (cartilage cells), chondrocyte progenitors and synoviocytes (cells lining the synovium of joints) are killed, directly or by apoptosis, by RBCs and their breakdown products. (Roosendall et al 1997; Hooiveld et al 2003; LaFeber et al 2008; Melchiorre et al 2017; Sethi et al 2018). Of special note, Hooiveld showed that in the presence of excess RBCs monocytes/macrophages give rise to an IL-1-mediated inflammatory response. The released IL-1 will cause local chondrocytes, as well as monocyte/macrophages, to produce hydrogen peroxide (H_2O_2). The peroxides mix with iron/ferritin, a breakdown product of hemoglobin from damaged RBCs, to produce hydroxyl radicals (·OH) (Hooiveld 2003; Jansen 2008). These hydroxyl radicals are extremely reactive and toxic and have been shown to kill chondrocytes directly as well as MSCs (Assmus 2010) and monocyte/macrophages.

    - The synthetic pathways that lead to proteoglycans, the glycoproteins that make up the lubricant lining of cartilage, are also permanently shut down by this induced local inflammation. (Kawase 2015; Cassano 2018; Castillo 2011; Amable 2013). Indeed, prolonged exposure (four days) of chondrocytes to whole blood leads to long-lasting inhibition of proteoglycan synthesis. Without the protective proteoglycans, osteoarthritis will follow.

- **Mesenchymal Stem Cells (MSCs)**
  MSCS are killed by ·OH produced by the local inflammatory response that is induced by RBCs. MSCs that survive exposure to ·OH are permanently disabled – they can no longer be recruited, differentiate, proliferate or release growth factors required in the repair process (Sethi 2018)

- **Monocyte Function**
  In the presence of RBCs, monocytes are pushed toward the M1 (pro-inflammatory) differentiation pathway. Just like platelets need to avoid RBCs in order to release

growth factors in a more physiologic way, monocytes need to avoid RBCs in order to activate directly into M2 macrophages (anti-inflammatory) and produce an anabolic/healing effect. In contrast, RBC contamination pushes the repair site towards and catabolic, non-healing environment.

In summary, RBCs do not facilitate tissue repair. Instead, they inhibit the process at just about every step mostly through the detrimental deposition of iron following hemolysis of RBCs and haemoglobin breakdown.

Looking at PRP red blood cell content in commercial systems, Harvest Smart PReP demonstrated the highest levels at 0.86 x 10^6/mm^3, followed by EmCyte Pure PRP A at 0.12 to 0.20 x 10^6/mm^3. All the other systems were at or below 0.05 x 10^6/mm^3. A stated above, the CAREprp System achieves a very low level of red blood cell contamination (>0.02 x 10^6/mm^3).

Therefore, CAREprp provides a unique opportunity to recover a high percentage of platelets while preserving the anabolic integrity of the cell sample.

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