A Prospective Single-donor Cellular Analysis of Two Different Autologous Platelet Rich Plasma Systems

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Abstract

Purpose: This study aimed to compare cellular contents of the DiscCath™ PRP System (New) to the EmCyte Pure PRP II (Historic) to determine if there was a statistically meaningful difference in platelet and white blood cell concentrations between these two point-of-care systems.
Methods: Prospective Single-donor cohort study at an outpatient interventional orthobiologics clinic involving 31 participants (17 male and 14 female) who supplied 64 distinct LR-PRP samples (one patient's PRP was analyzed on two separate treatment appointments). Baseline peripheral blood cell count data, and LR-PRP cell count data that included absolute platelet count increase (ABS PLT), platelet fold increase (PLT Fold), white blood cell counts and breakdown (WBC; LYM, MON, GRA), and hematocrit percentage (HCT).
Results: DiscCath™ produced a LR-PRP with a statistically higher ABS PLT (6.95 vs 5.65 billion platelets) and higher PLT Fold (15.92X vs 12.48X) when compared to EmCyte. DiscCath™ produced a LR-PRP with a higher ABS WBC (44.63 vs 41.86 million) and a lower HCT (10.30 vs 11.54 percent) when compared to EmCyte which did not achieve statistical significance.
Conclusion: The DiscCath™ PRP System concentrated platelets to significantly higher levels than the Emcyte PRP System. This may have clinical implications in the intradiscal treatment of patients with degenerative disc disease. Clinical studies are needed to assess the DiscCath™ PRP System to evaluate if these increases in platelet concentrations translates into improved clinical outcomes.

Keywords: Platelet-rich plasma, Intradiscal, Platelets, Systems, Single-Donor

Statements and Declarations

Funding: Regenerative SportsCare Foundation support

Competing Interests: Dr. Gregory Lutz is a coinventor of the DiscCath™ PRP System.

Author Contributions: M.M.S, C.K., and G.L all participated in study conception, study conduct, data interpretation, and manuscript preparation. All authors have read and approved the final manuscript.

Ethics Approval and Consent to Participate: This observational study was determined to be exempt by the IntegReview IRB (OBX-1005).

Introduction

Intradiscal platelet rich plasma (PRP) is an emerging novel regenerative therapy for patients with chronic low back pain (CLBP) from degenerative disc disease (DDD). There are now multiple clinical studies, including three randomized controlled trials, that have demonstrated different clinical outcomes despite studying similar patient populations.[1] [2] [3]One potential explanation for this phenomenon may be the type of PRP used, as well as, the dose of platelets administered. There are a wide variety of PRP systems with varying degrees of cellular content and the optimal platelet dosing and the ideal type of PRP for intradiscal use has not yet been established.

If we analyze the clinical outcomes studies heretofore that have been published we can ascertain some trends that may lead us to developing a potentially improved PRP system for intradiscal use.[4] Additionally, one of our first goals with intradiscal orthobiologics is patient safety. With the increased awareness of the role that bacteria play in disc degeneration an orthobiologic that takes this into consideration may have a beneficial therapeutic effect.[5] A recent review has suggested that a high platelet concentration leukocyte rich PRP may be the safest and lead to improvement in clinical outcomes in patients suffering from degenerative disc disease.[6] [7] [8] The concept being that we are potentially killing two birds with one stone: healing painful annular fissures while at the same time correcting the dysbiosis inside the disc that may contribute to chronic pain and degeneration.

Recent studies have suggested that higher platelet concentrations may lead to improved clinical outcomes.[9] [10] However, there are limitations of current commercially available point-of-care PRP systems to concentrate platelets to higher cellular levels.[11] Most systems concentrate platelets to three to five times baseline platelet counts, however, some systems can achieve ten times baseline.[12]

The DiscCath™ PRP system was specifically designed with an industry partner (Ranfac Corporation, Avon, MA) to potentially increase platelet concentrating abilities beyond what current commercial point-of-care systems are able to achieve. The concept being that higher concentrations of platelets would yield higher growth factor loads which may potentially improve clinical outcomes with intradiscal PRP procedures. The intent was to design a PRP point-of-care system that could consistently achieve a greater than fifteen times platelet fold increase that we could then take into new clinical trials to assess. Additionally, because of the risk of infection and the potential role that bacteria may play in disc degeneration the intent was to design a high concentration leukocyte-rich PRP (LR-PRP) system.

Thus the purpose of this study was to compare the cellular content of DiscCath™ PRP system to the PRP system (Emcyte Corporation) we used in our previous clinical study to assess whether or not this newer system could concentrate platelets and white blood cells to higher levels in a single-donor model.

Intradiscal OrthoBiologics are a promising treatment for degenerative disc disease in the lumbar spine. Physicians choose from a myriad of autologous injectable therapies with a variety of cell types, concentrations and preparation methods. The intradiscal orthobiologic with the most pre-clinical and clinical support is PRP[13]. Autologous PRP preparations are utilized in various orthopedic regenerative medicine applications to promote the stimulation and acceleration of bone and soft tissue healing. Currently there are a wide variety of proprietary kits available for physicians to choose from, to aid in processing the injectate. PRP preparations are believed to elicit a healing response through a paracrine mechanism which includes increased growth factor production and the recruitment of additional healing factors.

Current manufacturers indicate that a PRP concentration is at least five times the amount found in the baseline peripheral blood [11]. Several companies, including DiscCath™ Medical and EmCyte Corporation, have commercialized PRP preparation kits/protocols cleared for clinical use to ease preparation and help standardize the process. Such protocols afford clinicians a validated path to control the contents of the injectate, such as the inclusion or reduction of neutrophils or other populations of white blood cells (WBCs).

Efficacy of PRP for orthopedic regenerative medicine purposes has been documented consistently through recent literature, however a serious barrier to the advancement of the field is the lack of quality control and correlation between the content of PRP, the mechanism of action, and clinical outcomes. Two main factors contribute to the information gap. The first is the inherent variation in the preparation of PRP. Commercial kits employ proprietary variations of isolation methods (eg, centrifugation) and anticoagulant choice (eg, sodium citrate (SC) vs acid citrate dextrose solution A (ACD-A)), with each kit and/or method yielding a distinct product with varying levels of cell content and platelet activation [11, 14-17]. The second, is the gap can be attributed to a lack of content characterization of each PRP preparation on a point-of-care level during common clinical practice. This includes both basic characterization of cellular content, from analysis like complete blood counts (CBC), as well as more in-depth analyses of platelet functioning, including activation state and growth factor levels. In the literature, sufficient characterization of the blood samples was found to be completed in only 25.4% of studies using commercialized collection kits [11, 18]. This often leads to physicians if their preparation content conforms to the manufacturer's stated outcome during routine clinical use. Additionally, in practice, these kits are likely to have multiple end users within the same practice and are prepared from a widely varying patient demographic, all of which can introduce variation into the results. There have been many controlled studies garnering information on the yield of such preparations, however there is less information in the literature regarding the comparison of such kits during routine clinical use on the same patient in a less experimentally controlled setting [19-21]. Given the potential for variability in preparation and the current need to correlate cellular contents with therapeutic efficacy, it is critical for clinicians to understand the variation of the PRP system that they are using during routine clinical use [28].

There may be a relationship between platelet concentration and clinical outcomes of intradiscal PRP. Jain et al reported improvement in 80% of patients treated with intradiscal Leukocyte Rich PRP (LR-PRP) with an average of 2.75-x baseline platelet concentration. However, greater than 50% of patients who showed poor/no improvement had platelet concentrations in their PRP of less than 400,000 /uL [10]. Lutz et al recently published a retrospective intradiscal PRP study of higher concentration PRP (>10X platelet fold increase) and compared clinical outcomes to historic data from their initial 2016 DBRCT study (<5X platelet fold increase) [9]. They also showed improved clinical outcomes in those patients who received higher concentration intradiscal PRP (81% vs 56% patient satisfaction rate). Laboratory studies of PRP have demonstrated a linear relationship between growth factor content and platelet concentration [35].

The annulus fibrosus has been shown to respond to PRP-derived growth factors, which has limited volume capacity for injection [26]. Higher platelet concentrations in Intradiscal LR-PRP injections have been correlated with better patient outcomes and satisfaction [9]. There is evidence of an efficacy trend correlated with leukocyte rich vs. leukocyte poor preparations for intradiscal PRP. Several studies show inferior patient outcomes when using leukocyte poor preparations when compared to studies that used leukocyte rich preparations [2-3, 33]. A significant number of cases of spondylodiscitis have been reported with the use of leukocyte poor intradiscal PRP as well. Comparatively, to our knowledge, there are no known cases of spondylodiscitis associated with leukocyte rich intradiscal PRP [9, 27].

In our clinical practice, we routinely measure cell counts of all PRP systems used. In our most recent intradiscal PRP study we demonstrated that higher concentrations of platelets yielded improved outcomes using the DiscCath™ PRP Medical Platelet Separator 80ml kit (Codeveloped by DiscCath, LLC, New York, NY, and Ranfac Corporation, Avon, MA) and EmCyte Pure PRP II

60-mL kit (EmCyte Corporation, Fort Myers, FL) for PRP preparation. According to the manufacturer's instructions, both claim a greater than 8-fold enrichment over baseline for platelet counts. Both kits rely on the centrifugation method of preparation and have two protocols validated for the exclusion (Protocol A) or inclusion (Protocol B) of leukocytes.

For Intradiscal procedures we use a leukocyte-rich PRP (LR-PRP), for both safety and efficacy profiles. As mentioned above, there have been case reports of discitis associated with leukocyte poor PRP as well as with bone marrow aspirate [27]. There is evidence that intervertebral discs are colonized with significant levels of bacteria, most notably C. Acnes, which in recent review literature has been shown after antimicrobial treatments to result in back pain relief [5]. No cases of discitis have been observed within our patient population in over 12 years of practice performing intradiscal biologic injections when using LR-PRP [8]. To accommodate the volume limitations of intervertebral disc injections, we concentrate the LR-PRP preparation to 3 mL per level.

To properly administer a PRP with confidence in efficacy, concentration of cellular components should be within the therapeutic and manufacturer's standardized range [12]. The overarching hypothesis derived from commercialized PRP preparation is that each kit will yield clinical injectate contents as advertised by the manufacturer, and that practitioners choose which kit to utilize in practice according to the range specified by the manufacturer [22]. To test whether the above hypothesis is true, we performed a prospective chart review on CBC data obtained during medical treatment using the commercially available DiscCath™ PRP 80ml kit and the EmCyte PurePRP II 60-mL kit. We then compared the data obtained during clinical use to the output advertised by the manufacturers, with regard to platelet fold enrichment, hematocrit (%HCT), and enrichment or exclusion of granulocytes (GRA) through use of Protocol A or B. Please note that the GRA fraction is not further defined as neutrophils, eosinophils, and basophils in the following study; however, the GRA fraction is understood to be composed predominantly of neutrophil granulocytes as prepared by both the Cervos[14] and EmCyte system [24].

In addition, we hypothesized that leukocyte- rich preparations of each kit may still yield efficacious levels of total platelet cell counts for lower volume (3 cc) injections used in smaller anatomic sites of treatment, most notably intervertebral discs.

Materials and Methods

Patient Selection

This study involved the prospective analysis of 64 distinct PRP preparations from 31 consecutive patients, between the dates of December 9, 2021 and February 17, 2022. It was concluded that the scope of this study qualified for exempt review and retroactive patient consent was not required. All research and analyses were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its amendments. In terms of limitations, patient data was included for analysis given the following conditions: weight is greater than 110 lbs, non-pregnant, self-reported healthy, free of COVID-19, cold, and flu symptoms, and reports no history of prior infection within two weeks. For both pre- and post-injection, three-part differential CBCs were collected. The values from the performed CBCs were then stored and tabulated for data analysis.

Within the collection of these samples, the samples were then grouped based on which manufacturer's kit was used, with one group being designated towards the EmCyte system, and the other to the DiscCath™ kit.

Study Design

Primary Hypothesis

There will be a statistically significant difference in concentrations of the cellular components between the two PRP systems.

Participant Inclusion

Participants who received LR-PRP were excluded, and those who received PRP injections with leukocyte rich PRP were included from this trial. Additionally, patients who exhibited a low hemoglobin count (<10) and a lower platelet count (<100) were excluded from receiving the treatment.

Table 1. Analysis of Patient Demographics

Patient Demographics	
Number of Distinct Samples	64
Number of Patients	31
Age Range Distribution	**<29:** 1 **30-49:** 5 **50-69:** 17 **>70:** 8
Average Age	58
Gender Distribution	**M:** 17 **F:** 14

Study Protocol

Each kit used follows a standard protocol for preparation of injection, with this injection being prepared in accordance with manufacturer guidelines. For the EmCyte kit, 60 mL of blood is drawn from the patient and added to the apparatus, the first spin is performed at 3.8 rpm for 2 minutes, followed by decanting and transferring the upper layer along with its buffy coat to a new tube to be spun for another 3.8 rpm spin for 6 minutes. Following this, the platelets are then collected and injected into the targeted tissue.

The manufacturer's protocol for DiscCath™ is similar to that of EmCyte, except for the use of a specialized structure that is used with decanting, and a second spin at a higher rotation speed of 4.2 rpm. The sample collection would also start from a volume of 80 mL.

Before analyzing the samples in the hemacytometer, each sample was diluted at a 1:1 ratio with plasma in order to circumvent upper measurement limitations associated with the hemacytometer. The differences in numbers were then remedied by doubling the received result prior to tabulation.

Three samples are then collected, labeled "PRE", "POST", and "PPP". PRE corresponds to the initial concentrations of platelets and white blood cells in the first sample of whole blood collected. POST refers to the samples after concentration using either DiscCath™ or EmCyte technique prior to injection. PPP refers to a sample of the "Platelet Poor Plasma" that is left in the apparatus after injection.

The samples were then analyzed with a HORIBA ABX Micros ES60 hemacytometer, with a complete blood count being performed in order to tabulate the necessary parameters needed for comparison and analysis between the two systems.

From here the following parameters were isolated and recorded from the PRE and POST CBC results: PLT and WBC, with the WBC data being further broken down into lymphocytes (LYM), monocytes (MON), and granulocytes (GRA). The data would then undergo significant analysis to draw conclusions about differences between the two kits.

Results

Table 2. Mean whole blood characteristics pre injection from 34 patients

Average Whole Blood Concentrations	
	Mean
Platelet concentration ($x\ 10^6/ml$)	176.94 ± 48.23
White blood cell concentration ($x\ 10^6/ml$)	4.87 ± 1.69
Lymphocyte concentration ($x10^6/ml$)	1.52 ± 0.61
Monocyte concentration ($x\ 10^6/$	0.32 ± 0.16

ml)	
Granulocyte concentration ($x\ 10^6/ml$)	3.03 ± 1.30
Pre injection hematocrit (%)	37.95 ± 10.79

As evidenced in Table 1, the study was allowed to proceed with the 34 selected patients as their platelet concentration and white blood cell concentration numbers were within normal and healthy ranges.

DiscCath™ PRP System vs. EmCyte PurePRP II Evaluation

For the comparison of the two systems, several parameters were derived from the accumulated data. Outside of the aforementioned raw data consisting of PRE and POST samples, calculations were then performed to determine the percentage increase in both white blood cells and platelet counts, this data was then labeled as "[Parameter] Fold Increase". The equation to calculate the fold increase using WBC Fold Increase as an example is as follows:

$$WBC\ Fold\ Increase = \frac{(WBC\ Post) - (WBC\ Pre)}{WBC\ Pre} * 10$$

Additionally, in order to compare the total number of platelets injected between the two systems, Absolute Platelet Count was calculated. The calculation was performed with the sample equation as follows, with the constant *2.5* representing the amount of solution injected, as 3 mL injectates were prepared, with 0.5 mL being saved for analysis:

$$Absolute\ PLT\ Count = (2.5 * 10^6) * (PLT\ Post)$$

Following the evaluation of these values, the primary focus then became comparison, so in order to compare the two systems, a question was posed regarding the most appropriate statistical test to perform. As two different populations with unequal variances are being compared, a two tailed T-test assuming unequal variances was performed in order to delineate any significant statistical differences.

Furthermore, additional breakdowns for leukocyte data were collected, and then divided into Lymphocytes (LYM), Monocytes (MON), and Granulocytes (GRA), with the same calculations and evaluations being performed. Post injection Hematocrit percentages were also measured to ensure that both systems' values stayed within the manufacturer's indicated range.

Statistical Analyses Performed

T-tests for the analysis parameters were performed assuming unequal variances, this is because the two systems used are inherently different and from different manufacturers, so assuming equal variances in this case would be unfeasible. For this series of tests, a standard confidence interval of 5% was used.

Based on the observed P values, the parameters in which P<α are "Post injection platelet concentration". "Platelet fold increase", "Absolute platelet count", and "Lymphocyte fold increase". For the other parameters, no statistically significant difference was observed. In a previous paper on the topic of intradiscal, it is observed that there exists a direct correlation between granulocyte count and C acne recovery, which then illustrates the importance of maximizing granulocyte levels within procedure.

Tables

Table 3. Statistical summary of average PRP characteristics obtained from analysis

	PRP Preparation System		
	DiscCath™ Platelet Separator 80mL	EmCyte Pure PRP II 60mL	P-value (α=0.05)
Post injection platelet concentration	2784.44 ± 1041.44	2261.25 ± 483.10	0.0139 *

$(x\ 10^6/\ ml)$			
Platelet fold increase (X)	15.92 ± 6.63	12.48 ± 4.04	0.0152 *
Post injection white blood cell concentration $x\ 10^6/\ ml)$	44.63 ± 19.07	41.86 ± 14.93	0.5254
White blood cell fold increase (X)	8.67 ± 3.68	8.22 ± 3.78	0.6335
Absolute platelet count ($x\ 10^9$)	6.95 ± 2.60	5.65 ± 1.21	0.0139 *
Post injection lymphocyte concentration ($x\ 10^6/\ ml)$	27.11 ± 11.30	22.47 ± 7.46	0.0589
Lymphocyte fold increase (X)	18.5 ± 9.15	14.54 ± 4.25	0.0314 *
Post injection monocyte concentration ($x\ 10^6/\ ml)$	5.39 ± 3.01	4.57 ± 1.81	0.1984
Monocyte fold increase (X)	17.06 ± 8.79	14.95 ± 6.33	0.2778
Post injection granulocyte concentration ($x\ 10^6/\ ml)$	12.48 ± 7.45	15.37 ± 7.44	0.1292
Granulocyte fold increase (X)	3.50 ± 3.29	4.24 ± 1.92	0.2744
Post injection hematocrit (%)	10.30 ± 4.88	11.54 ± 3.53	0.2499

Platelet Concentrations

Initially, both analyzed systems delivered platelets to the targeted region while maintaining a positive fold increase, indicating that platelet levels above the baseline were injected. Comparing the two systems together, it is evident that based on T-test analysis that DiscCath™ maintains statistical significance to the EmCyte system with a reported P-value of 0.0139 in terms of absolute platelet counts (Fig. 1, 5), and a P-value of 0.0152 in terms of the fold increase. Additionally, on average, DiscCath™ (2784.44 ± 1041.44 $x\ 10^6/ml$) Delivered approximately 23% more platelets than EmCyte (2261.25 ± 483.10 $x\ 10^6/ml$) into the injection site.

White Blood Cell Concentrations

There were no statistically significant differences between DiscCath™ and EmCyte in terms of white blood cell changes. While the P-value was not below the alpha value and it was therefore impossible to codify the difference between the two systems, it was observed that DiscCath™ (44.63 ± 19.07 $x\ 10^6/\ ml$) delivered approximately 6% more WBCs than EmCyte (41.86 ± 14.93 $x\ 10^6/ml$) into the injection site.

Leukocyte Characterization and Composition Comparison

The collected WBC data was then broken down further into specific lymphocytes, monocytes, and granulocytes (Fig. 2-4).

From these numerical observations, statistical significance was found in the lymphocyte fold increase associated with DiscCath™ (P=0.0314), and was also close to statistical significance in the absolute number of lymphocytes delivered (P=0.0589). DiscCath™ (27.11 ± 11.30 $x\ 10^6/ml$) delivered approximately 20% more lymphocytes than EmCyte (22.47 ± 7.46 $x\ 10^6/ml$) into the injection site.

There were also no statistically significant differences between the two systems in terms of monocytes (P=0.1984) and granulocytes (P=0.1292). DiscCath™ (5.39 ± 3.01 $x\ 10^6/ml$),

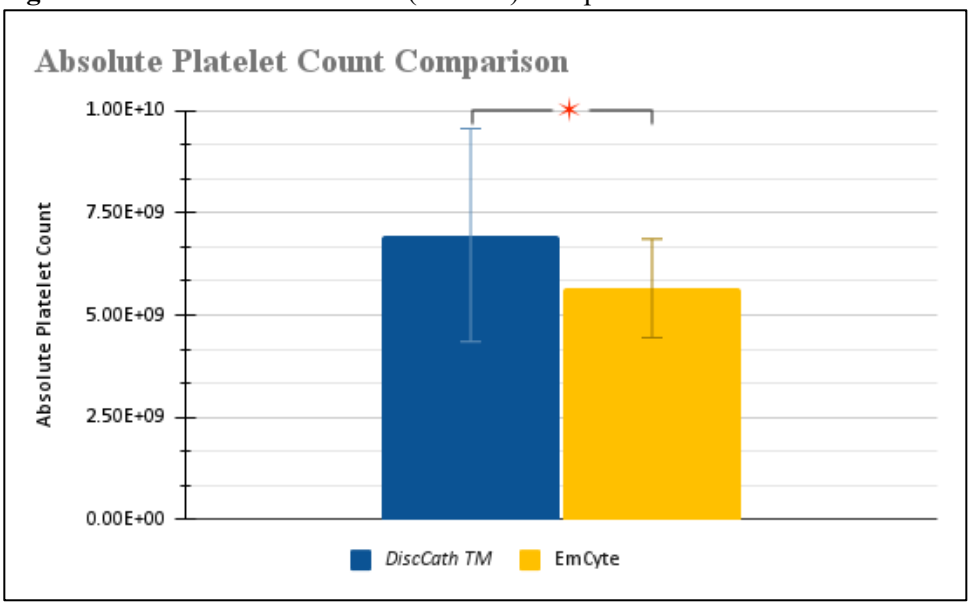
on average, delivered approximately 17% more monocytes than EmCyte ($4.57 \pm 1.81\ x\ 10^6/\ ml$). Alternatively, EmCyte ($15.37 \pm 7.44\ x\ 10^6/ml$) produced approximately 23% more post injection granulocytes than DiscCath™ ($12.48 \pm 7.45\ x\ 10^6/ml$).

Hematocrit

DiscCath™ (10.30 ± 4.88 %) maintained a post injection hematocrit that was approximately 10% lower than that of EmCyte (11.54 ± 3.53 %), with a lower hematocrit being advantageous due to it being an indicator of more platelets, white blood cells, and growth factors within the sample.

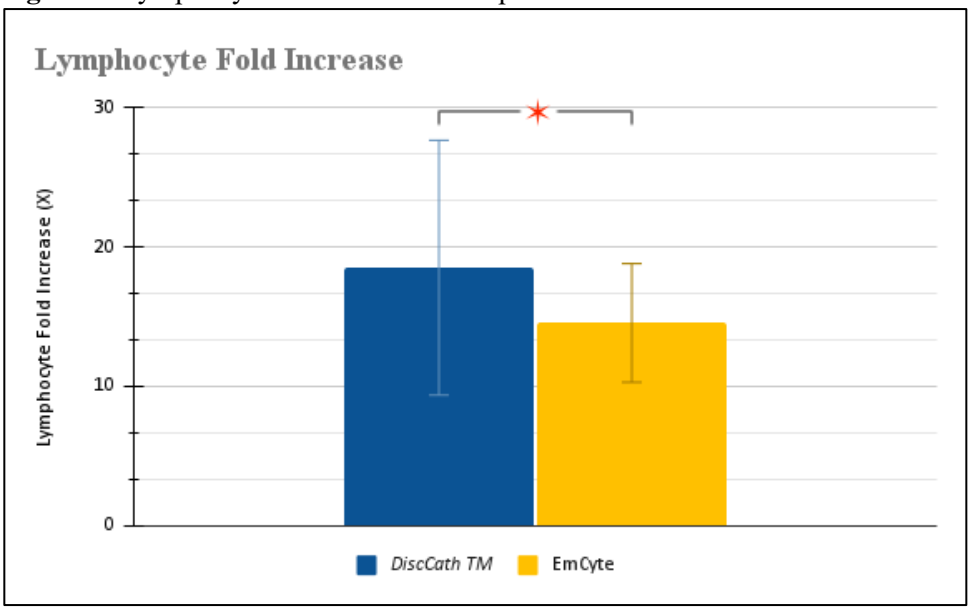
Graphs and Visualizations of Aforementioned Findings

Figure 1. Absolute Platelet Count (Billions) Comparison



*Assuming α=0.05

Figure 2. Lymphocyte Fold Increase Comparison



*Assuming $\alpha=0.05$

Figure 3. Monocyte Fold Increase Comparison

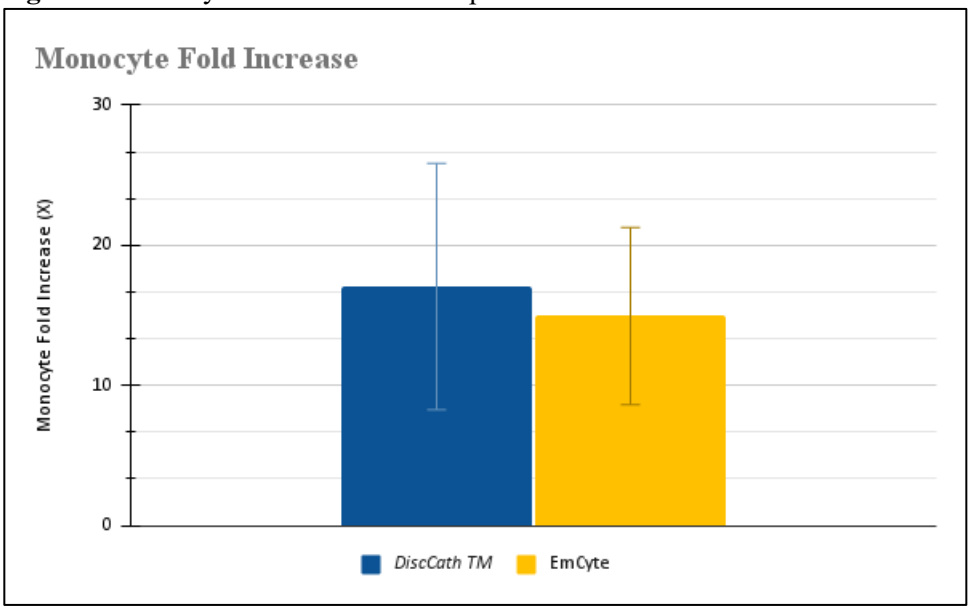


Figure 4. Granulocyte Fold Increase Comparison

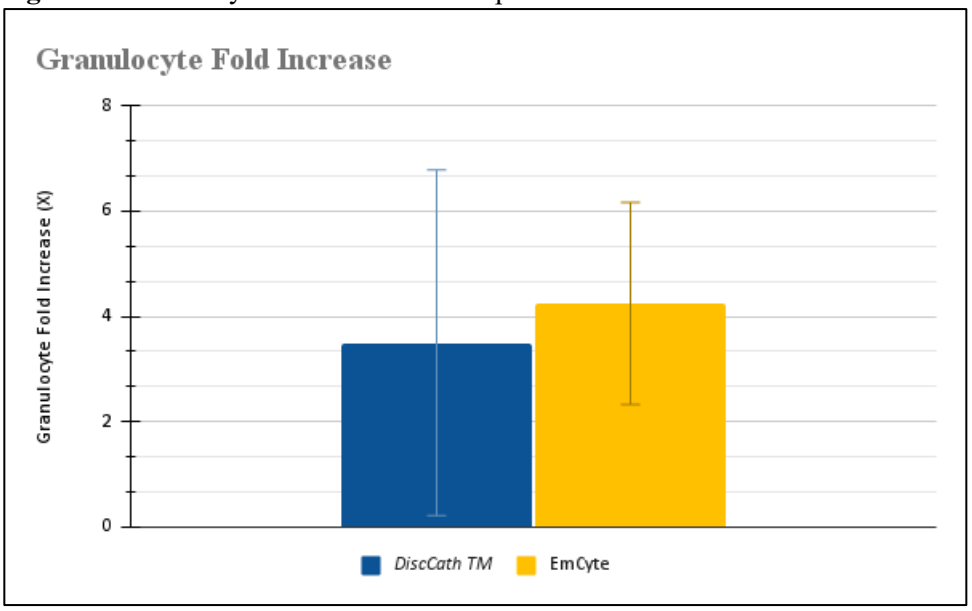
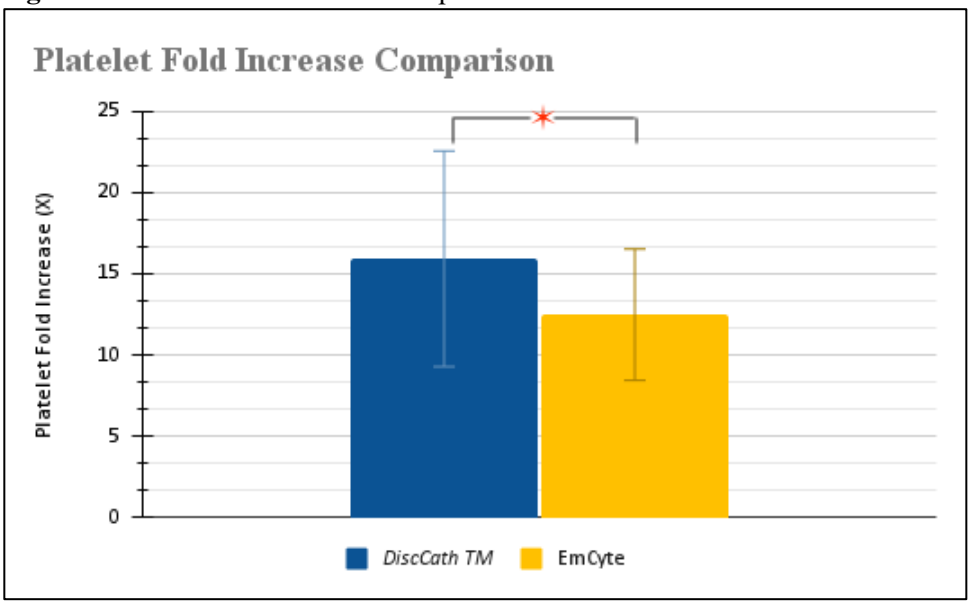


Figure 5. Platelet Fold Increase Comparison



*Assuming $\alpha=0.05$

Safety

There were no reported complications associated with both DiscCath™ and EmCyte.

Discussion

Physician confidence in the choice of a PRP kit over other comparable kits is based on each manufacturer's reported contents, particularly because CBC analysis on PRP preparations at point-of-care is uncommon in clinical practice. The characterization of PRP kits during clinical use is critical to confirm the content of the injectate, which may coincide with a kit's efficacy in potential for tissue healing [9, 12, 27-28]. What gets measured can be better managed to improve the quality control of the PRP product. This will hopefully lead to improvement in clinical outcomes of intradiscal biologic therapies.

The current study highlights several important points about the comparative performance of the DiscCath™ Platelet Separator 80ml kit versus the EmCyte PurePRP II 60-mL kit during routine medical use outside of a controlled experimental setting. Our data provide a clear view of the cellular makeup of both kits using the leukocyte-rich preparation method in a non-experimental setting, with statistically significant increases in platelet concentrations. The DiscCath™ system produced a mean platelet fold increase of 15.92-x over baseline, compared to the Emcyte System which produced a mean platelet fold increase of 12.48-x over baseline. Previous comprehensive reviews of commercial kits provide 5 to 9-x fold increases above baseline [32] which both DiscCath™ and Emcyte exceed by a significant margin. Of the leukocytes which were noticeably enriched, lymphocytes had an increase of 19-x over the baseline, monocytes 17-x over the baseline, and granulocytes 3-x over the baseline. Both kits consistently produced on average a >8-fold increase in PLT concentration during routine clinical use, confirming both manufacturer's reported concentration levels. For reference, the original determinations by the manufacturer Emcyte were performed on a Coulter Ac-T diff 2 Hematology Analyzer and for DiscCath™ on a Horiba ABX Micros ES60 [23-24]. Thus the performance conformed to expected values for both PLT and GRA enrichment parameters under conditions of routine clinical use.

The %HCT levels reported by EmCyte for the device are <1% for Protocol A and < 20% for Protocol B [25]. Our data confirms that Protocol B reproducibly returned a %HCT of <20% during routine clinical use. The %HCT levels reported by DiscCath™ for the device are HCT < 20% for Leukocyte Rich Protocol [25]. Our data also confirmed that Protocol B returned a %HCT of <20%. These patients also had a higher-than-expected GRA enrichment of 3-fold.

The findings also revealed that our internal protocol volume modification of 3 mL for intradiscal use resulted in a significant increase in mean absolute counts of PLTs, LYMs, and GRAs, but differences in monocytes were not significant between the two volumes. The reason for this is unknown.

Widespread characterization of the injectate at point-of-care is the crucial first step toward fully elucidating the mechanism of action for PRP in regenerative medicine applications. Further evaluation of the therapeutic window of PRP may allow for a simple qualitative assessment for clinicians to use prior to injection with the intention of significantly improving patient outcomes. The need for specific data on cell count content is further discussed below.

The data derived from this study elucidates statistically significant differences in platelet concentration efficacy between two commercially available PRP systems. In the two systems studied, DiscCath™ proved superior in providing consistently higher PLT concentrations. The Emcyte system had less variability, yet lower mean platelet concentrations per milliliter of injectate. In clinical practice, this study indicates that when physicians are making modifications in injectate volumes for smaller anatomical sites of treatment, they may do so with confidence in substantial increases in PLT concentration and absolute PLT counts. This alone is a significant numerical advantage in terms of platelets delivered and potential improvement in growth factor delivery in small volume sites of injection, such as within the intradiscal space. Defining the absolute platelet level of an injectate in relation to patient improvement is difficult, however ensuring substantial enrichment is necessary to the viability of these procedures. Further characterization is needed to bridge this gap of knowledge. Studying patient outcomes compared to the absolute platelets and leukocytes received per injection may lead to greater understanding in platelet levels associated with efficacy.

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