Analysis of PEAK™
Platelet Rich Plasma System

A pre-clinical evaluation of device performance

Principal Investigator: Dr. Robert Mandle, Ph.D.

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767c Concord Ave.
Cambridge, MA 02138

The study presented in this document has been conducted and validated by Dr. Robert Mandle Ph.D. at BioSciences Research Associates, Inc.
Analysis of PEAK™ Platelet Rich Plasma System

IN VITRO TESTING

SUMMARY:

The PEAK Platelet Rich Plasma System recently introduced by DePuy Synthes Mitek Sports Medicine is an innovative system for preparation of a concentrated platelet product from a small volume of whole blood. Twenty seven ml of whole blood produces approximately 3.3 ml of platelet concentrate containing an average of 4.1 billion platelets. The range of total deliverable platelets from 60 donor samples was 2.7 billion to 6.5 billion platelets. Platelets and mononuclear leukocytes were concentrated efficiently while granulocytes were not. Red blood cells were significantly reduced. The neutral pH of 7.3 in the concentrate eliminates requirements to add bicarbonate for pH adjustment prior to administration. The PEAK system requires minimal work surface (5” x 5”) and has a total processing time of less than 3 minutes, using only 2 aseptic entries to minimize risk of product contamination. There is a high operator safety profile due to needle-free processing and isolation of the liquid medical waste within the disposable.

INTRODUCTION:

The PEAK Platelet Rich Plasma (PRP) System recently introduced by DePuy Synthes Mitek Sports Medicine has a lightweight (415 gm (<1 pound)) and small (12 x 12 cm (~5 inches square)) foot-print centrifuge. Utilizing a unique disposable and high speed, fixed angle centrifugation, it is capable of separating blood components in less than 1 min and the total processing time is less than 3 min. The system design eliminates the need for a counterbalance as required in conventional centrifuges; the waste plasma and red blood cells are retained in and disposed of with the device. This study evaluated the performance of the PEAK PRP System and the quality of the PRP product produced from 60 healthy human whole blood samples.

EXPERIMENTAL DESIGN:

This is a single center study conducted by BioSciences Research Associates, Inc. (BSR). All studies were conducted within BSR’s Quality Systems and are cGMP compliant. BSR has extensive experience with development and testing of platelet concentration devices and product evaluation, including support for FDA CBER and CDRH filings.

Human whole blood was obtained from each donor following informed consent. All blood collection protocols were approved by the New England Institutional Review Board. Donors met the requirements of the American Association of Blood Banks (AABB) and the FDA CBER. There were no specific exclusion specifications, other than the donor was to be healthy. There was no selection for age, sex or ethnicity. Blood was drawn by syringe into anticoagulant (final concentration of 10% Anticoagulant Citrate Dextrose Solution, Formula A (ACD-A)) according to the manufacturer’s Instruction for Use. Thirty ml of anticoagulated blood samples were processed to produce approximately 3.3 ml of PRP product within approximately 1 hour of receipt from phlebotomy. The PRP was then divided into 2 aliquots. One aliquot was analyzed at 0 hour (immediately following processing) and the second was analyzed at 4 hours post-processing. A 4 hour delay was chosen because it represents a realistic worst case time period that the PRP might be held following processing and prior to use in a procedure. Processing was carried out according to the manufacturer’s Instructions for Use.

The parameters listed in Table 1 were analyzed to evaluate the PRP product.

Table I: Analysis Parameters

<table>
<thead>
<tr>
<th>Product Parameter</th>
<th>Method</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet yield</td>
<td>Automated Hematology Analyzer</td>
<td>Efficiency of platelet capture from the whole blood sample</td>
</tr>
<tr>
<td>Platelet concentration factor</td>
<td>Automated Hematology Analyzer</td>
<td>The relative change in platelet concentration over the concentration in whole blood</td>
</tr>
<tr>
<td>White blood cell (WBC) concentration factor</td>
<td>Automated Hematology Analyzer</td>
<td>The relative change in white blood cell concentration over the whole blood sample</td>
</tr>
<tr>
<td>Red blood cell (RBC) concentration factor</td>
<td>Automated Hematology Analyzer</td>
<td>The relative change in red blood cell concentration over the whole blood sample</td>
</tr>
<tr>
<td>Resting platelet activation</td>
<td>Flow Cytometry</td>
<td>Measure of the activation state of the platelets in the product following processing</td>
</tr>
<tr>
<td>Platelet viability</td>
<td>Flow Cytometry</td>
<td>Measure of platelet response to activating stimulus</td>
</tr>
<tr>
<td>WBC differential</td>
<td>Automated Hematology Analyzer</td>
<td>Determines the type of white blood cells and their relative concentration in the product</td>
</tr>
<tr>
<td>Product pH</td>
<td>Blood Gas Analyzer</td>
<td>Determines the pH of the product</td>
</tr>
</tbody>
</table>
RESULTS:

Hematology Analysis:

Figure 1 summarizes the relative concentration efficiency of the PEAK System for Platelets, Leukocytes and Red Blood Cells. On average, 75% of total platelets are recovered, leading to a ~7-fold increase in platelet concentration. Leukocytes were concentrated less efficiently (See Figure 1). The final red blood cell concentration in the platelet concentrate was reduced almost 3 fold compared to the starting blood sample, whereas leukocytes were concentrated approximately 5 fold.

White Blood Cell Differential:

There is a change in the percentage of granulocytes and mononuclear cells in the PEAK System PRP compared to the whole blood samples. As shown in Figure 2, the majority of leukocytes in whole blood are granulocytes with mononuclear cells in significantly lower numbers. The reverse is seen in the PEAK System PRP.

Platelet Activation:

P-selectin (CD62p) is an integral membrane cell adhesion protein of the platelet alpha granule that is responsible for mediating platelet adhesion to damaged vessel walls, and platelet interaction with leukocytes to modulate their activity at sites of injury. P-selectin becomes expressed on the surface of the platelet when stored growth factors are released from the platelet alpha granules. In vitro, expression of surface p-selectin in response to a platelet agonist is an indicator of platelet function and correlates with growth factor release. Platelet surface P-selectin was measured on resting whole blood samples and on PEAK System PRP as well as on samples stimulated with a platelet agonist adenosine diphosphate (ADP). The data is summarized in Figure 3 for 0hr whole blood and PRP samples.
pH:
The pH of the PEAK System PRP was measured with a Radiometer ABL90 blood gas analyzer. The mean pH was 7.32 +/- 0.07 for time 0 samples and 7.33 with a standard deviation of 0.06 for time 4 hour samples.

Stability of PEAK System PRP up to 4 Hours post processing:
Analysis of PEAK PRP samples was done at the completion of the whole blood sample processing and again after storing the sample at room temperature for 4 hours. Table II compares the 0 and 4 hour samples for the key parameters.

Table II: PEAK PRP Stability (Mean and Standard Deviation)

<table>
<thead>
<tr>
<th>Test Parameter</th>
<th>At completion of processing</th>
<th>4 hour post processing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Count</td>
<td>1253 ± 254</td>
<td>1250 ± 239</td>
</tr>
<tr>
<td>Resting Platelet P-Selectin</td>
<td>7.3 ±4.5</td>
<td>9.1 ± 4.0</td>
</tr>
<tr>
<td>ADP Stimulated Platelets</td>
<td>98 ±0.9</td>
<td>97 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.3 ± 0.07</td>
<td>7.3 ± 0.07</td>
</tr>
</tbody>
</table>

DISCUSSION:
The PEAK System reliably produced a consistent platelet rich plasma product across 60 normal donors with an average platelet yield of 75% and a platelet concentration ~7X that of starting whole blood. There was no evidence of clinically significant platelet activation during processing with the PEAK System, and excellent platelet viability and function were evident as demonstrated with p-selectin analysis by flow cytometry on resting and ADP stimulated platelet samples. Growth factor analysis was not conducted as part of this study; however robust induction of P-selectin surface expression following ADP activation suggests strong platelet degranulation and attendant growth factor release.

Leukocyte concentration in the PEAK System PRP is approximately 5 times that of starting blood samples resulting in a recovery averaging 59%. Mononuclear cells are preferentially retained (84% recovery) as compared to granulocytes (43% recovery). The mononuclear cells include monocytes and lymphocytes and other cells of similar size and density including CD34 positive progenitor cells. Mononuclear cells can supply additional growth factors including vascular endothelial growth factor (VEGF). Granulocytes, the majority of which are neutrophils, contribute to anti-microbial activity and wound site debridement.

In contrast to mononuclear cells that are long-lived and contribute to extended release growth factors, neutrophils normally undergo programmed cell death (apoptosis) within 1-2 days, and therefore do not contribute to extended release growth factor pools. Because extended release growth factors are thought to be beneficial in PRP, it is more desirable to preferentially concentrate mononuclear cells in leukocyte rich PRP preparations.

Red Blood Cells (RBCs) are not concentrated by the PEAK System; 96% of the RBCs are not recovered, resulting in a PRP product with ~3-fold less RBC concentration compared with whole blood.

CONCLUSION:
The innovative use of vertical centrifugation with a unique and proprietary disposable design in the PEAK System allows for the extremely efficient separation of whole blood into its component parts with minimal process-induced platelet activation and excellent platelet viability. This process results in a PRP that remains stable for at least 4 hours after preparation, insuring an optimal delivery of growth factors. The centrifugation process separates red cells, buffy coat and plasma within one minute with a total processing time of approximately 3 minutes. The centrifuge weighs less than 1 pound with a 5 inch by 5 inch footprint. The system design eliminates the need for a counterbalance and retains waste red blood cells and plasma for safe efficient disposal. The PEAK System resulted in high platelet concentration (~7 X baseline) from 27 ml of blood and the pH of the PRP product remained within the normal pH range of whole blood.

Funding Source: This report summarizes data funded by DSM Biomedical (Exton, PA), manufacturer of the PEAK Platelet Rich Plasma System.