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Comparison of Methods for Point of Care Preparation of Autologous Platelet Gel

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Abstract: A platelet gel (PG) is produced by the addition of calcium chloride and thrombin to a platelet concentrate (PC). PG releases multiple growth factors, which have the ability to initiate and stimulate one growth factor's function in the presence of others. This finding has resulted in the use of PG in orthopedic, plastic, and reconstructive surgery. The study compared the commercial systems available for the preparation of PG. All procedures were performed according to the manufacturers directions. The devices were evaluated with respect to ease of use, collection efficiency, platelet quality, and growth factor release. The SmartPRep® requires only four processing steps compared to 12 to 24 required by other devices. The SmartPReP® and the CATS® were the most reproducible, as evidenced by their low coefficient of variation of 13% and 16%. The mean platelet yield was 72% for the SmartPReP®, 58% for the 3iPCCS, 54% for the Sequestra®, 31% for the Secquire®, 31% for the CATS®, 27% for the Interpore Cross®, and 42.6% for the Biomet GPS™. The mean total amount of PDGF-AB and TGF-B1 obtained from the SmartPReP® is greater than other systems evaluated. The SmartPReP® produced a consistent PC with a yield that was four times baseline range with the lowest coefficient of variation. **Keywords:** platelet gel, platelet yield, growth factors. *JECT. 2004;36:28–35*

Most platelet preparations are used for transfusion in an effort to prevent or treat bleeding caused by thrombocytopenia. Platelets can also be used locally as a source of growth factors that play an essential role in wound and bone healing and in bone regeneration (1–4).

The body naturally heals damaged tissue at an injury site in a single manner that is well described and referred to as the "healing cascade". The initial stages of this progression are clot formation, inflammation, and cell proliferation; these processes are controlled by proteins carried in platelets, white blood cells, and plasma. Specifically, clot formation involves the interaction of multiple proteins in the plasma and activated platelet membranes. Inflammation involves the migration of macrophages and white blood cells to the injury site and is controlled by proteins that are released from white cells and platelets. Cell proliferation and cell division to replace damaged and destroyed cells is really a combination of two cellular activities. The first is the migration of mesenchymal stem cells from surrounding healthy tissue into the injury site. The second is triggering these cells to divide. The proteins that control cell migration and cell division are carried in the platelets and white blood cells. These proteins are referred to as growth factors, and their mechanism of action has been described, and their contribution to cell proliferation is well known (5–7).

The ultimate purpose in preparing a platelet concentrate and, subsequently, platelet gel for local application, is to maximize the availability of the multiplicity of proteins at the injury site. The methods of preparation can directly affect the characteristics of the product. The purpose of this study was to compare seven commercial technologies for the preparation of an autologous platelet concentrate for local application.

MATERIALS AND METHODS

Donor Selection

The institutional review board for the Center for Blood Research approved the blood collection protocol. Blood was obtained from normal healthy donors (males and females) who met the blood donor requirements of the American Association of Blood Banks (AABB) and the FDA-CBER. Each donor served as his/her own control during the study. The cell salvage systems (CATS® and Sequestra®) required the collection of a unit of blood from

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the donor. This precluded using the same donors to evaluate all of the devices.

Platelet Collection and Identification

Each donor was identified by number, which would enable a reviewer to identify and compare the test results. All procedures were performed according to the detailed instructions provided by the manufacturer.

Systems Evaluated

1. SmartPReP® (Harvest Technologies, Plymouth, MA distributed by DePuy, Warsaw, IN as Symphony). The system can use one or two dual chamber disposables. Whole blood is added to the first chamber, and the centrifuge is activated. The red blood cells (rbcs) and the platelet-rich plasma (PRP) are separated. The PRP is automatically decanted into the second chamber and concentrated by centrifugation. A portion of the platelet-poor plasma (PPP) is removed and the platelets resuspended in the remaining plasma (7–10 mL). The newest version of this system, SmartPReP®2, has a floating shelf and will process 22.5 to 60 mL of anticoagulated whole blood per disposable, yielding a concentrate of 3 mL or 7 to 10 mL. Two 60-mL disposables can be processed simultaneously if 20 mL of PC are required.
2. Sequestra® (Medtronics, Minneapolis, MN). The Sequestra is an intraoperative salvage device that uses the classic Latham bowl technology to separate PRP from a unit of whole blood.
3. CATS® (Fresenius, Wilmington, DE). This system is an intraoperative blood salvage device that is a modification of their apheresis (platelet collection) system. Processing requires the collection of a unit of whole blood.
4. Ultraconcentrator (Interpore Cross®, Irvine, CA). This system is a hollow fiber hemoconcentrator that utilizes 60 mL of the product obtained from an intraoperative salvage device. A specific workstation must be used to provide housing for the syringes, hemoconcentrator and air flow. During our evaluation, the Sequestra was used to provide the platelet product.
5. PCCS® (3i, Palm Beach Gardens, FL). This is a miniaturization of a double pack blood collection system used in blood collection facilities. Following the two-centrifugation steps, the transfers from a primary to a secondary pack require the injection and removal of specific volumes of air through designated entry ports. The system provided by the manufacturer provides only a single disposable. A volume of 60 mL of anticoagulated whole blood can be processed.
6. Secquire® (PPAI Medical, Fort Myers, FL). This system utilizes a modified 50 mL plastic centrifuge tubes. The caps of the tubes have modified entry ports that have plastic inserts that must be removed when loading the system with whole blood and subsequent removal of PPP and platelet concentrate (PC). Two different centrifugation steps are required to complete the process. Two disposables can be processed simultaneously.
7. GPS™ (Biomet Co., Warsaw, IN). This technology utilizes a modified flat bottom 60-mL plastic centrifuge tube. The test tube contains a buoy and an internal coiled device located in the tube cap, which must be lowered to remove the PPP following centrifugation. Manufacture instructions require that sterile gloves must be worn to maintain sterility of the product. The volume of buffy coat (BC) that can be removed is not adjustable (±5.0 mL).

Evaluations Performed

The assays performed on the donor blood samples and the prepared platelet products as well as the purpose of each test are summarized in Table 1. The qualitative assays performed to evaluate platelet function and viability are the same assays performed on platelet products prepared for transfusion. To evaluate the effect of the secondary ultraconcentration process of the Interpore Cross® system, samples were evaluated following separation with the Sequestra® (prefiltration) and after passage through the hemoconcentrator (postfiltration).

Statistics

When provided, statistical comparisons were performed using the Student's *t*-test for paired comparisons (two tailed). Differences were considered to be significant if $p < .05$. Means and standard deviations (SD) for at least five samples ($n = 5$) are reported unless otherwise noted.

RESULTS

Processing Details

Table 2 compares the operating parameters of each of the systems evaluated. The SmartPReP® has the least number of processing steps (5), the shortest total processing time (13 minutes) and the least investment in operator time. The salvage devices (CATS® and Sequestra®) require a unit of blood for processing. All other systems evaluated, except for the Interpore Cross® device require only 50–60 mL of whole blood for processing. The Interpore Cross® requires 60 mL of the platelet product from a salvage device for processing. The number of sterile barrier entries is similar for all the devices. However, neither the Secquire® or the GPS™ have entry ports that can be prepped as per the accepted hospital practice. The Secquire® has a removable plastic plug during processing, and the GPS™ mandates the use of sterile gloves. In an unrelated study, we have cultured the SmartPReP® PC aerobically and anaerobically throughout an 18-day period with negative results.

Comparison of the Platelet Products

Table 3 compares the characteristics of the platelet

Table 1. Quantitative and qualitative analyses performed.

Tests Performed	Samples Evaluated	Method	Test Purpose
Complete blood counts	Baseline/post-processing	Automated coulter (Coulter Max-M)	Quantitative assay: Counts used to assess overall collection efficiency and resulting platelet counts in final product.
P-Selection	Post-processing	Flow cytometric assay	Qualitative assay used to assess platelet viability: Measurement of the P-selectin glycoprotein of unstimulated platelets investigates the preservation of platelet activity (lower values indicate less platelet activation). Increased expression of P-selectin following exposure to a platelet agonist such as ADP demonstrates that platelet viability is maintained.
Platelet aggregation	Post-processing	Optical aggregometry following exposure to collagen agonist (200 μg/mL)	Qualitative assay used to assess platelet function assessed by measuring the extent of platelet aggregation when a sample of the platelet product is exposed to a physiologic platelet activator.
Albumin	Baseline/post-processing	1. Radio immunodiffusion (The Binding Site Inc., San Diego, CA) 2. Chemical assay	Albumin used as a marker for efficiency of protein collection measured using: 1) an antigenic quantitative assay (radio immunodiffusion) and 2) qualitative chemical assay.
Fibrinogen	Baseline/post-processing	1. Radio immunodiffusion (The Binding Site Inc., San Diego, CA) 2. Clotting assay (Klaus technique)	Test selected to: 1) assess fibrinogen levels in final product (antigenic quantitative assay) and, 2) evaluate the quality/function of the available fibrinogen (qualitative assay) in the plasma supernatant of the prepared platelet products.
Fibronectin	Baseline/post-processing	Radio immunodiffusion (The Binding Site Inc., San Diego, CA)	Quantitative antigenic assay to measure the levels of fibronectin in the plasma supernatant of the prepared platelet products.
Adhesion molecules	Post-processing	ELISA (R&D systems)	Quantitative antigenic assay of Stem Cell Factor (SCF) and Soluble Vascular Adhesion Molecule-1 (sVACAM-1) in the plasma supernatant of the prepared platelet products.
Growth factors	Post-processing*	ELISA (R&D systems)	Quantitative antigenic assay of PDGF-AB and TGF-β_1.

*Quantitative analysis of growth factors performed following platelet activation with 1000 U/mL of calcified bovine thrombin.

product produced by the various systems. It is important to note that the SmartPReP®, 3i PCCS®, Secquire,® and GPS™ can all simultaneously process an additional disposable. The PC volume of the SmartPReP® can be adjusted from 7–10 mL by varying the volume of the PPP supernatant for resuspension of the PC by utilizing specific spacers. The 3i PCCS™ and Secquire® PCs are fixed by the process at 8–10 mL. The GPS™ produces a ±5 mL platelet concentrate (see Table 7). The newest modification of the SmartPReP® can use either a 60-mL or a 20-mL disposable. The latter produces a 3.3 mL PC (see Table 7).

The SmartPReP® system, as shown in Table 3, has the greatest percentage yield (72%) and the most reproducible product as demonstrated, having the lowest coefficient of variation (CV = 13%). The percentage yield is a measure of the system's efficiency in isolating platelets from a volume of whole blood processed. CV is a measure of the device's capability to produce a consistent product reliably. The low CV values for both the SmartPReP® and the CATS® should be expected because both systems are automated. However, the CATS® has a mean platelet yield of only 31%. The mean platelet yield for the 3i PCCS® was 58%, which is comparable to the mean yield obtained by blood banks for a random donor platelet concentrate (obtained from a unit of whole blood). However, the markedly elevated CV demonstrates that the system does not produce a consistent product. For the Sequestra® to produce a platelet yield comparable to a random donor platelet concentrate, multiple bowl cycles are required.

Table 2. Processing details of systems evaluated.

System	Anticoagulated Blood Processed (mL)	Sterile Barrier Entries	Number of Processing Steps	Operator Time (min)	Process Time (min)	Total Time (min)
*SmartPReP® $n = 25$	60	5	4	2	13	15
3i PCCS® $n = 10$	60	5	24	15	17	32
Secquire® $n = 5$	50	6	12	10	12	22
CATS® $n = 5$	450	4	16	10	10	20
Sequestra® $n = 5$	450	4	20	10	30	45
†Interpore Cross® $n = 5$	60	3	15	10	15	25
GPS™ BIOMET	60	7	23	15	12	27

*All systems were compared to SmartPReP® using a split donor sample.
†Utilized 60 mL of Sequestra product.

The Interpore Cross® system had the lowest level of measurable platelets, even though it was volume reduced by 2.6-fold. Passage through the hollow fiber hemoconcentrator resulted in platelets that were fragmented, clumped, and/or bound to the hollow fibers. The end product is a platelet releasate rather than a viable platelet concentrate.

Another useful measure of a PC product is the relative increase in platelet count above the baseline platelet count in the whole blood sample. Only the SmartPReP® and the CATS® produce a product that is at least four times baseline.

Evaluation with Respect to Platelet Viability

The in vitro indicators of platelet viability and function used in transfusion medicine are P-selectin and platelet aggregation. These markers have also been used to examine platelet activation during preparation (8–10). A brief description of these tests is presented in Table 1. The results of our studies are shown in Tables 4 and 5. During the latter phase of our studies, we modified our technique for P-selectin using a double antibody technique, which has high sensitivity for the detection of minor changes in surface antigen density that occur during platelet activation. This technique was used to compare the SmartPReP® with the Secquire® (Table 5).

The P-selectin values for the SmartPReP®, CATS®, 3i PCCS®, and the Secquire® are similar. The Sequestra® had slightly elevated levels of P-selectin, probably because of the multiple bowl fills during product preparation. The P-selectin expression of the PCs prepared by these devices increased following challenge with the agonist ADP, indicating that platelet viability is preserved. P-selectin expression for the Interpore Cross product without ADP was significantly greater than for the other systems ($p = .018$). P-selectin expression for the Interpore Cross product, with and without the agonist ADP, were identical (42 ± 14% and 43 ± 27%, respectively), indicating that there was no residual stimulatory activity.

The CATS® and SmartPReP® have the same mean aggregation response. This is to be expected because the CATS® is a modified platelet apheresis device, and the SmartPReP® has a step that prevents the formation of platelet aggregates. All other systems have visible platelet aggregates that will dispense upon standing. This probably accounts for their low normal aggregation response. Platelet aggregation of the Interpore Cross product was significantly impaired as compared to the other systems ($p = .001$). Platelet aggregation of the Interpore Cross samples were impaired by the ultraconcentration process as demonstrated by the low post-filtration aggregation response, mean 17 ± 21% as compared to the prefiltration response of 75 ± 4% (Sequestra®).

Fibrinogen, Albumin, and Adhesion Molecules

During the course of this study, we evaluated the fibrinogen levels using both an antigenic assay and by the Klauss technique (functional assay used in coagulation laboratories). The data for three of the systems are shown in Table 6. The levels of fibrinogen, quantified, using the antigenic assay, was significantly greater in the Interpore

Table 3. Comparison of the platelet products.*

System	Baseline Platelet Count $\times 10^3/\mu L$	Platelet Conc. Vol-mL	Platelet Conc. Count $\times 10^3/\mu L$	Platelet Yield (%)	Platelet Increase over Baseline	CV (%)
SmartPReP®	251 ± 55	9.3 ± 1.8	1016 ± 389	72 ± 10	4.0	13
3i PCCS®	268 ± 59	8.2 ± 1.2	978 ± 367	58 ± 22	3.6	38
Secquire®	220 ± 13	9 ± 0.5	348 ± 194	31 ± 15	1.6	48
CATS®	239 ± 46	29 ± 0.6	992 ± 162	31 ± 5	4.1	16
Sequestra®	225 ± 33	83 ± 6	582 ± 128	54 ± 11	2.6	20
†Interpore Cross®	582 ± 128	23 ± 0.6	356 ± 233	27 ± 22	0.6	81

*All values are mean ± SD.
†Utilizes 60 m of Sequestra product – volume reduction 2.6×.

Table 4. In vitro indicators of platelet viability.

System	*P-Selectin (%) Mean ± SD		†Aggregation (%) Mean ± SD
	No ADP	With ADP	With Collagen
SmartPRep®	13 ± 8	36 ± 12	80 ± 9
3i PCCS®	16 ± 10	37 ± 9	78 ± 7
CATS®	9 ± 3	31 ± 2	82 ± 9
Sequestra®	29 ± 9	40 ± 8	75 ± 4
Interpore Cross®	43 ± 27	42 ± 14	17 ± 21

*Normal value 10–20%.
†Normal value 81 ± 12%.

Table 5. In vitro indicators of platelet viability.

	*P-Selectin % Mean ± SD		†% Aggregation Mean ± SD
	No ADP	With ADP	With Collagen
SmartPRep® $n = 3$	6.31 ± 1.88	55.5 ± 5.7	80 ± 2.0
Secquire® $n = 6$	5.31 ± 0.87	49.2 ± 5.2	69 ± 1.8

*Normal value 12 ± 4%.
†Normal value 81 ± 12%.

Cross platelet product than in the Sequestra® or SmartPReP® PC. This would be expected and is directly related to the ultraconcentration process used in preparation of the Interpore Cross® product. Although the antigenic assay determines the total concentration of fibrinogen in the sample, the Klauss technique quantifies the level of clottable or functional fibrinogen. The level of clottable fibrinogen in the Interpore Cross® product (postfiltration) was significantly less than the value using the antigenic assay ($p = .007$). The differences were not observed with the platelet products prepared by the Sequestra® or SmartPReP® or in random sampling of the 3i PCCS®. Only 69% of the fibrinogen in the Interpore Cross® product is clottable.

As part of the study, plasma albumin levels were used as a marker to quantify protein collection. Plasma albumin levels as measured in a clinical chemistry laboratory in all platelet products, except for the Interpore Cross®, were similar to the baseline donor value (with a mean of 3.4 ± 0.6 g/dL. As expected, this was lower than in the Interpore Cross® product (mean value: 7.5 ± 1.35 g/dL), which involves a secondary ultraconcentration process to remove plasma water. The antigenic assay for albumin in the Interpore Cross® product had a mean value of 9.1 g/dL. These findings support the previously described fibrinogen results. There was no difference observed between the chemical or antigenic assay for albumin in the Sequestra® platelet product.

The levels of fibronectin and adhesion molecules in the PCs of all the systems are similar (data not shown). The ultrafiltration step of the Interpore Cross® process concentrates the plasma proteins by removing water, which results in a higher concentration of the attachment proteins (fibronectin and adhesion molecules) in the PC plasma. However, the Interpore Cross® product has a significantly higher hematocrit because less plasma is available, the total amount of the various attachment proteins is comparable for the devices.

Table 6. Effect of ultraconcentration on fibrinogen levels.

	Clottable Fibrinogen (mg/dL) Mean ± SD		Antigenic Fibrinogen (mg/dL) Mean ± SD	
	Baseline	Product	Baseline	Product
SmartPReP®	254 ± 64	259 ± 69	240 ± 65	246 ± 72
Sequestra®	254 ± 64	247 ± 72	240 ± 65	255 ± 74
Interpore Cross®	247 ± 72	386 ± 16	255 ± 74	558 ± 120

Growth Factors

Three of the systems (CATS®, Sequestra®, Interpore Cross®) require seven times more blood for the initial platelet separation than that required by the other devices evaluated. The volume of the PCs for three of these systems (SmartPReP®, 3i PCCS®, Sequire®) is ±10 mL; whereas, that of the GPS™ is only ±5 mL. To account for the differences in blood processing volumes, and the volume of the PC, the total amount of growth factor released was compared equalizing the volume of blood processed for each system to 60 mL. The data are shown in Figure 1. Given the same quantity of blood processed, the mean total amount of PDGF-AB and TGF-β-1 released is slightly greater for the SmartPReP® than the 3i PCCS® but significantly greater than the other systems evaluated ($p > .05$).

DISCUSSION

Autologous platelet concentrate combined with thrombin and calcium has been used to prepare a platelet gel that releases biologically active proteins stored in platelet vesicles and granules. In contrast to recombinant growth factors, autologous PCs and, subsequently, platelet gel modulates and upregulate one growth factor's function in the presence of a second or third. Recombinant growth factors focus only on a single generation pathway. This source of autologous growth factors and other proteins can contribute to the healing process. As documented in oral–maxillofacial, spinal, and plastic surgery, as well as in the treatment of chronic wounds (1–4).

The ultimate objective in preparing a platelet concentrate at point of care is to maximize the availability of this multiplicity of proteins directly at the injury site. A recent study by Haynesworth et al. concluded that a dose response could be demonstrated for the effect of platelet gel on the migration and proliferation of human mesenchymal stem cells (hMSC) (11). This study supports previous stud-

Table 7. Comparison of the SmartPReP®2 with Biomet GPS™.*

Anticoag. Blood Processed mL	Platelet Count $\times 10^3/\mu L$	Platelet Concentrate Vol-mL	Platelet Concentrate Count $\times 10^3/\mu L$	Platelet Yield %	CV %	Platelet Increase Over Baseline
SmartPReP®2 22.5 mL $n = 10$	244 ± 38	3.3 ± 0.1	1083 ± 233	65 ± 9.0	14.0	4.4 ± 0.6
SmartPReP®2 60 mL $n = 10$	241 ± 38	10 ± 0.12	1151 ± 164	80 ± 3.4	4.2	4.8 ± 0.2
SmartPReP®2 60 mL $n = 4$	221 ± 36	7 ± 0.3	1675 ± 88	86.8 ± 3.4	4.0	7.6 ± 0.3
Biomet GPS™ 60 mL $n = 6$	192 ± 48	5.5 ± 0.2	809 ± 396	42.6 ± 9.0	21.0	4.1 ± 0.8

*If SmartPReP®2 product volume was the same as the GPS™ (5.5 mL), the platelet increase over baseline would be 9.6.
†All values are mean ± SD.

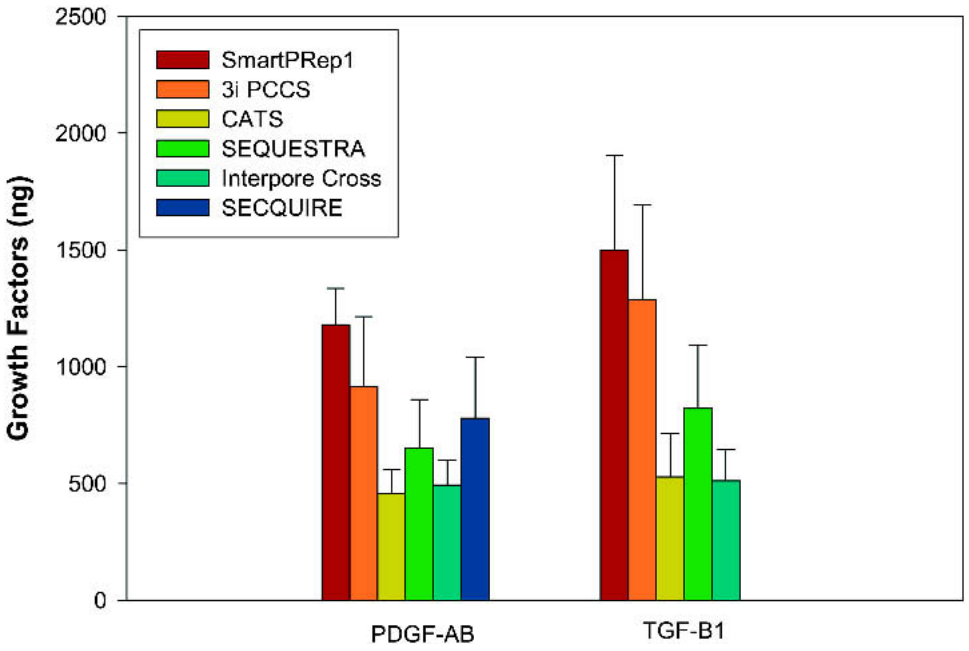

Figure 1. Total growth factors released from platelet concentrate preparations. All systems adjusted to 60 mL processed volume.

ies that have used platelet concentrates to demonstrate that the delivery of growth factors from platelets' secretory granules is dependent upon the binding of fibrinogen to a platelet membrane integrin (12,13). It is, therefore, critical that the method of platelet concentrate preparation optimizes protein availability. As demonstrated by the study results, the presently available systems for the point of care preparation of a platelet concentrate have very significant differences. The clinician needs comparative information to decide which system is most likely to offer the best protein product reliably and consistently. The results of this study address the need for standardization of preparative methods and from the point of view of transfusion medicine and molecular biology, the characterization of the components obtained.

The differences in the end product are, for the most part, because of the volume of blood collected and the method of platelet concentration used by the systems evaluated. As shown in Table 2, three of the systems require a unit of blood (400–500 mL) for processing. Many patients undergoing major surgery predeposit several units of blood, which is often done up to 72 hours before surgery. A collection of only 60 mL or less of whole blood would be more acceptable to most anesthesiologists. The SmartPReP® is the most automated system, requiring only 2 minutes of operator time and one-third of the processing steps required by the other devices. Thus, there is virtually no increase in the workload of the often over-burdened operating room personnel. Two of the devices (Secquire® and GPS™) do not have entry ports that can be sanitized in keeping with standard hospital practice.

The overall platelet collection efficiency is shown in Table 3. The SmartPReP® has the greatest percentage yield (72%) and is the most reproducible, as demonstrated by having the lowest coefficient of variation, only slightly under that of CATS®. However, the efficiency of the SmartPReP® system allows it to produce a comparable product to the CATS, with much less blood and without the need for a specially trained dedicated operator. The platelet collection efficiency was lowest for the Interpore Cross® system, and it was the least reproducible (CV 81%). Following filtration, the volume of the Interpore

Cross® product was reduced from 60 mL to a mean of 23.4 mL. One would have expected a 2.6-fold increase in platelet counts had the platelets remained intact or were all recovered. This observation is attributable to platelet activation resulting in platelet adhesion to the surface of the hollow fiber membrane; a well-documented phenomenon associated with the use of hollow fibers for hemoconcentration and dialysis (14). The 3i PCCS® has a platelet yield of 58 ± 22%, which is comparable to the yields obtained when preparing a random donor platelet concentrate from a unit of whole blood. However, the system has a markedly elevated CV of 38%. The preparation of autologous platelet concentrates at point of care is objected to by many individuals in transfusion medicine because of the concerns of qualitative reproducibility. The low CVs of both automated systems should put this objection to rest.

Platelet aggregation and P-selectin are two standard assays used to evaluate the quality and function of platelets (8–10). Platelet aggregation evaluates the ability to function (aggregate) when exposed to a physiologic platelet agonist (e.g., collagen). The alpha-granule membrane protein, P-selectin, is sequestered on the internal membrane of the alpha-granule on resting platelets. Expression of P-selectin is, therefore, an indicator of the extent of platelet activation with larger values of P-selectin correlating with a greater degree of activation. The Interpore Cross system has a markedly reduced platelet aggregation (17%) and a high value for direct P-selectin (without ADP-43%). The failure to obtain an increase in P-selectin in the Interpore® Cross PCs following the administration of ADP indicates that those platelets are completely activated and, therefore, not viable (Table 4). In comparison, the values observed for P-selectin and platelet aggregation for the other systems tested demonstrate that platelet function and viability are preserved to varying degrees and consistent with platelets prepared for transfusion. Normal values for collagen-induced aggregation of platelets prepared for transfusion is 81 ± 12% and that for direct (unactivated) P-selectin is 12 ± 4%.

A surprising observation during the course of this study was the fact that only 69% of the fibrinogen in the Interpore Cross® product was clottable. The level of fibrinogen certainly is sufficient to produce a stable clot. A similar discrepancy was observed when the plasma albumin was measured by both a chemical and antigenic assay. The existing in vitro growth factor measurements are immunologic assays, not functional assays. It is, therefore, not unreasonable to assume that only a portion of the growth factor levels measured in the Interpore Cross® product are functional.

Previous studies from our laboratory have determined that there is a direct correlation between platelet concentration and growth factor release (15). Figure 1 presents a comparison of the total ng of growth factor released by each of the systems when adjusted to a whole blood processed volume of 60 mL. The mean growth factor levels or "protein load" in the final product was slightly greater for the SmartPReP® than the 3i PCCS® and significantly greater than the other systems evaluated. However, the data on the coefficient of variation would indicate that only the SmartPReP® can confidently reproduce these levels.

There are two studies that have correlated the increase in platelet count above baseline to a positive in vivo effect. The Marx study described an increased bone regeneration based on a platelet concentrate mean level of 3.4 times baseline (1). The work of Haynesworth et al. demonstrated that an increase of five times baseline provided a significant increase in chemotactic and mitogenic stimulation of cultured hSMCs (11). This confirmed the work of Slater et al., who demonstrated that the addition of viable human platelets (prepared by a blood bank) added to the culture medium stimulated proliferation of human osteoblast-like cells as much as 36-fold, as compared with the controls. Based upon the required concentration of platelets in a unit prepared from 500 mL of whole blood (5.5×10^{10}) and the amount of platelets he used, this would be equivalent to four to five times baseline (12).

Two new systems have recently become available for use in the operating room. The preliminary results obtained in our laboratory, comparing the platelet products, are shown in Table 7. The Smart PreP-2™ system contains a floating shelf of a specific density to separate the buffy coat and uppermost layer of red cells from the concentrated red cells during centrifugation. The system will process either 22.5 or 60 mL of anticoagulated whole blood. The GPS™ system contains a buoy and an internal device consisting of a plunger and tubing to remove the PPP and, subsequently, the buffy coat following centrifugation. The volume of the product is set at ± 5 mL, which is obtained from 60 mL of anticoagulated whole blood.

CONCLUSION

There are several devices available for point of care preparation of autologous platelet concentrates and, subsequently, platelet gel. Our studies comparing the different devices demonstrated variations in both quality and quantity. The SmartPReP® produced a consistent PC in the four times baseline range, with the lowest coefficient of variation (13%) while requiring only a small volume of blood. This finding is not unexpected because the SmartPReP® is the most automated system. Elimination of manual manipulation, as would be expected, does result in reduced variability. The ability to produce a PC that is four to five times baseline consistently should result in a protein load to enhance wound healing and bone regeneration based on the currently available in vitro and in vivo data.

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REFERENCES

1. Marx R, Carlson E, Eichstaedt RM, et al. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg Oral Med Oral Pathol. 1998;85:638–46.
2. Lowery G, Kulkarni S, Pennisi AE. Use of autologous growth factors in lumbar spine fusion. Bone. 1999;25:47s–50s.
3. Adler SC, Kent KJ. Enhancing wound healing with growth factors. Facial Plast Surg Clin N Am. 2002;10:129–46.
4. Patel T, Sethi P, Miranda J, et al. The use of platelet concentrate in posterolateral fusion: Biomechanical and histological analysis. Presented at the 28th Meeting of the International Society for the Study of Lumbar Spine, Edinburgh, Scotland, June 2001.
5. Andrian U, Mackay C. T-cell function and migration. N Engl J Med. 2000;343:1020–34.
6. Kubes P, Ward P. Leukocyte recruitment and the acute inflammatory response. Brain Path. 2000;10:127–35.
7. Lindemann S, Tolley ND, Dixon DA, et al. Activated platelets mediate inflammatory signaling by regulating interleukin 1B synthesis. J Cell Biol. 2001;154:485–90.
8. Murphy S, Rubella P, Batolini F, et al. In vitro assessment of the quality of stored platelet concentrates. Transfus Med Rev. 1994;8:29–36.
9. Rinder H, Ault K. Platelet activation and its detection during the preparation of platelets for transfusion. Transfus Med Rev. 1998;12: 271–87.
10. Schmitz G, Rothe G, Andrew R, et al. European working group on clinical cell analysis: Consensus protocol for the flow cytometric characterization of platelet function. Thromb Haemost. 1998;79:885–96.
11. Haynesworth SE, et al. Mitogenic stimulation of human mesenchymal stem cells by platelet releasate suggests a mechanism for enhancement of bone repair by platelet concentrate. Presented Orthopedic Res Soc, March 2002.
12. Slater M, Patara J, Kingham K, et al. Involvement of platelets in stimulating osteogenic activity. J Orthop Res. 1995;13:655–63.
13. Gfatter R, Spaenger B, Boeck A, et al. The mitosis of fibroblasts in cell culture is enhanced by binding GPllb-llla of activated platelets on fibrinogen. Platelets. 2000;11:204–14.
14. Tsunoda N, Kokubo K, Saika K, et al. Surface roughness of cellulose hollow fiber dialysis membranes and platelet adhesion. ASAIO J. 1999;45:418–23.
15. Kevy S, Jacobson M. Preparation of growth factor enriched autologous platelet gel. Trans Soc for Biomaterials 27th Meeting. April 2001, Seattle, WA.