

Database Review of Possible Factors Influencing Point-of-Care Platelet Gel Manufacture

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Abstract: The application of autologous platelet gel (APG) to surgical wounds has been advocated during the last 10 years to speed bone and wound healing, minimize infection, and modify postoperative pain. There are few data available to confirm these claims. Prior to organized study, consistency and agreement in manufacture of the APG is needed. In this study, an attempt is made to isolate factors that are important in developing a consistent gel. A database review was performed to determine factors that affect PG quality and function. Quality was assessed by platelet count and fibrinogen concentration, whereas function was assessed by an arbitrary scale of gelling, in which a “1” was equal to a soup texture, “3” equal to a “Jell-O” consistency, whereas a grade of “2” was somewhere in between. Data specific to the blood draw and platelet-rich plasma sequestration were collected. Two hundred and sixty cases of APG production were reviewed. The volume of blood drawn was dependent on the

machine used. Gelling of the APG was independent of platelet count and fibrinogen concentration. Gels that were rated soupy had an average platelet count of $540 \text{ K}/\mu\text{L} \pm 327 \text{ K}/\mu\text{L}$ and a fibrinogen concentration of $225 \text{ mg}/\text{dL} \pm 76 \text{ mg}/\text{dL}$ whereas a firm gel had an average platelet count of $486 \text{ K}/\mu\text{L} \pm 264 \text{ K}/\mu\text{L}$ and a fibrinogen concentration of $229 \text{ mg}/\text{dL} \pm 78 \text{ mg}/\text{dL}$. The manner in which the blood was drawn along with the site from which the blood was drawn influenced the platelet concentration of the platelet-rich plasma. Optimal platelet counts were obtained when blood was drawn from a peripheral vein and sequestration was performed with the Medtronic Sequestra or the Medtronic Magellan. Ultimate gelling of PG is independent of fibrinogen and platelet concentrations. This work suggests a need for further research into the manufacture of APG. **Keywords:** platelet gel, quality, fibrinogen concentration, fibrin glue. *JECT. 2004;36:250–254*

Autologous platelet gel (APG) was developed in the early 1990s as a byproduct of platelet-rich plasma (PRP) sequestration in cardiac surgery (1,2). This gel was advocated as an alternative to fibrin glue. Since that time, extensive interest in the use of APG has been generated. Advocates of platelet gel feel that this gel has benefits that include an increase in bone and wound healing, a decrease in postoperative infection, a decrease in postoperative pain, and a decrease in postoperative blood loss. Few of these benefits have been demonstrated clinically, but anecdotal reports are abundant (3,4). The outcome studies that do exist primarily show benefits of APG use in oral and maxillofacial surgery (5–7), and cosmetic surgery (8–10).

Platelet gel is manufactured by mixing PRP with thrombin and calcium. This gel is then applied topically to a wound surface. The PRP can be manufactured either through standard blood bank procedures, or it can be manufactured through point-of-care devices, which include cell-salvage equipment or tabletop devices. Unlike standard blood bank procedures, the point-of-care devices have no well-defined protocols and quality standards by which the PRP is manufactured. The lack of standardized protocols has been previously discussed by Zimmermann et al. (11) and an accompanying editorial (12).

Our institution’s perioperative autotransfusion service has been receiving an increasing number of requests from our surgeons asking for APG. The expectation of the surgeon is that the product possesses a gelatin-like consistency. The service has faced inconsistency in gelling of our APG; therefore, a database was created to evaluate factors that might relate to this gelling. From the database review presented, it is hoped that knowledge might be

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gained towards developing a standardized method of producing PRP in the operating room environment. Until a standardized, repeatable method is developed, clinical outcome studies, which can be generalized to all populations, are difficult.

MATERIALS AND METHODS

Following institutional review board approval, a database was created to track cases performed by the autotransfusion service. These cases include cell salvage, normovolemic hemodilution, and plasma and platelet sequestration. All cases performed by the service are documented. Data specific to platelet sequestration includes the patient's name, age, sex, weight, presurgical hematocrit and platelet counts, a presurgical fibrinogen count, the machine used for platelet production, the volume of blood drawn to produce the PRP, the duration of the blood draw, the site of the blood draw, the PRP volume, the PRP platelet count, the PRP fibrinogen concentration, and the consistency of the final gel as reported by the surgeon. The consistency of the gel is graded by the surgeon upon application with a scoring system, with "1" equal to a soup texture, "3" equal to a "Jell-O" consistency, whereas a grade of "2" was somewhere in between.

Equipment

Four types of machines were used for the production of PRP. Two cell-salvage devices (COBE BRAT II, COBE Cardiovascular Inc, Arvada, CO; and the Medtronic Sequestra, Medtronic Inc., Minneapolis, MN) and two table-top devices (Symphony System, DuPuy Acromed, Raynham, MA; and the Medtronic Magellan, Medtronic Inc., Minneapolis, MN) were used. Each device was used according to manufacturer recommendations for production of PRP. The COBE protocol was altered so that the fill speed was 100 mL/min and the spill speed was 100 mL/min. When the cell salvage devices were used, the PRP was collected until a red flare was seen in the collection bag. Generally, the cell-salvage devices are used when both cell salvage and platelet gel is requested. The table-top devices are used in surgical procedures where minimal blood loss is anticipated.

Phlebotomy

Blood was drawn from either a peripheral vein, an indwelling central venous catheter, or through an indwelling arterial line. When PRP is to be manufactured using a cell-salvage device, blood is drawn into a standard CPDA-1 donor bag using gravity. To assure that the volume of blood drawn is appropriate for the amount of citrate in the donor bag, a scale is used to measure the weight of the blood. Periodic manual agitation of the blood was performed to assure blood/citrate mixing. The

amount of blood drawn for fractionation was dictated by the machine used. For the BRAT II, the blood volume drawn was according to the patient's starting hematocrit. For the Sequestra, 450 mL of blood was drawn.

Gel Production

At the time of application, the PRP is transferred to the surgical field into a sterile covered container. A second container is filled with the platelet activator consisting of a calcium/thrombin mix that is made by mixing 10 mL of 10% calcium chloride with the 5000 units of lyophilized bovine thrombin. Just before application, 7 mL of PRP is drawn into a 10-mL syringe. When ready to place the APG on the wound, 1 mL of the calcium-thrombin mixture is added to the syringe. Inverting the syringe 180° three times allows for optimal mixing. The gel is then applied to the surgical wound in a fashion dependent on the type of surgery.

Statistics

One-way analysis of variance with a Bonferroni post-hoc test was used when comparisons between three groups was made. Linear regression was used when an association between two variables was tested. Pearson's correlation was used to assess correlation between the patient's platelet count and the gel platelet count. A p value > 0.05 was considered statistically significant.

RESULTS

Two hundred and sixty cases of gel production were retrieved in the database, of which 68% were spine procedures, 29% were plastic surgery cases, and 2% were total knee replacements. From these cases, 25 cases had incomplete data. Where applicable, data from these 25 cases was incorporated into the analysis. Table 1 shows comparison data across each machine. Patients were comparable in weight, starting hematocrit and platelet count, and their ASA status. The cell-salvage equipment needed significantly more blood than the table top devices but generated larger volumes of PRP. The Sequestra and Magellan manufactured the product with the highest platelet count and the highest fibrinogen concentration (Figure 1).

The quality of the platelet product was thought to relate to the duration of the blood draw, the volume of the blood draw, and the site from which the blood was drawn. A downward trend was seen in platelet counts with longer draw times; however, the slope of the regression line was not significantly different than zero ($p = 0.0908$; Figure 2). The lack of significance may relate to few data points in the prolonged blood draw period. The site from which the draw was made did influence platelet count with significantly higher platelet concentrations when the blood was drawn from a peripheral or central vein vs. an arterial

Table 1. Comparison across four methods of producing platelet rich plasma.

	BRAT II (n = 40)	Sequestra (n = 66)	Symphony (n = 103)	Magellan (n = 45)
Patient age	52.2 ± 16.2	51.4 ± 14.0	54.4 ± 17.0	58.2 ± 10.8
Patient weight†§	80.4 ± 20.2	84.8 ± 19.7	77.4 ± 20.7	68.1 ± 12.0
Patient HCT	40.8 ± 4.4	36.3 ± 4.6	36.3 ± 5.3	38.4 ± 6.0
Patient PLT count	255.8 ± 62.7	255.1 ± 72.4	254.2 ± 91.6	284.0 ± 85.1
ASA	2.5 ± 0.06	2.3 ± 0.6	2.4 ± 0.6	2.3 ± 0.7
Patient fibrinogen	249.3 ± 76.6	261.7 ± 125.1	256.5 ± 102.3	269.7 ± 107.2
Vol. of blood drawn*†‡§¶	549.0 ± 64.5	456.6 ± 78.8	107.6 ± 18.2	111.3 ± 21.1
Duration of draw*†‡§¶	18.6 ± 16.7	12.6 ± 8.6	7.8 ± 5.8	7.8 ± 3.2
Gel volume*†‡§¶¶	48.5 ± 18.3	38.9 ± 9.5	22.1 ± 6.3	16.7 ± 3.2
Gel PLT count†‡§¶	407.7 ± 205.9	605.6 ± 342.5	437.5 ± 240.5	657.7 ± 265.6
Gel fibrinogen	202.4 ± 56.7	222.0 ± 75.6	225.5 ± 79.2	244.7 ± 116.2
Gel consistency#§¶	2.5 ± 0.7	2.5 ± 0.7	2.6 ± 0.7	2.0 ± 0.3

HCT, hematocrit; PLT, platelet; Vol., volume.

**p* < 0.05 for Sequestra vs. Magellan; †*p* < 0.05 for Sequestra vs. BRAT; ‡*p* < 0.05 for Sequestra vs. Symphony; §*p* < 0.05 for Magellan vs. BRAT; ¶*p* < 0.05 for Magellan vs. Symphony; #*p* < 0.05 for BRAT vs. Symphony.

line. Platelet counts averaged 394 K/μL ± 180 K/μL, 679 K/μL ± 318 K/μL, and 591 K/μL ± 301K/μL when drawn from an arterial line, peripheral vein, or a central line, respectively (Figure 3). No correlation was found between the patient’s platelet count and the gel platelet count (*r* = 0.156).

When evaluating the role of platelet count on gel consistency, it appeared that there was no relationship between high platelet counts and the resulting surgeon report of the gelling. Likewise, no relationship existed with fibrinogen concentrations. Gel which was rated soupy had an average platelet count of 540 K/μL ± 327 K/μL and a fibrinogen concentration of 225 mg/dL ± 76 mg/dL, while a firm gel had an average platelet count of 486 K/μL ± 264 K/μL and a fibrinogen concentration of 229 mg/dL ± 78 mg/dL (Figure 4).

DISCUSSION

The development of platelet gel originated as an attempt to produce an autologous equivalent to fibrin glue. From this origin, an expectation has arisen that the platelet gel product should produce a firm coagulum. Thus, a “good” gel has been deemed one that quickly forms a firm coagulum. Whether a good coagulum is necessary for the intended outcome improvement has not been demonstrated and warrants evaluation.

A lack of predictability in gel quality as manufactured by our service led us to evaluate the relationship between platelet and fibrinogen concentrations and a crude index of gel quality. From this review, no association between a good quality gel and the platelet count was seen, nor was there an association seen between the gel quality and fibrinogen concentration. These data would suggest that a measure of platelet function might be necessary to predict whether a PRP will form a good coagulum. In addition, directed questioning of the patient prior to surgery may

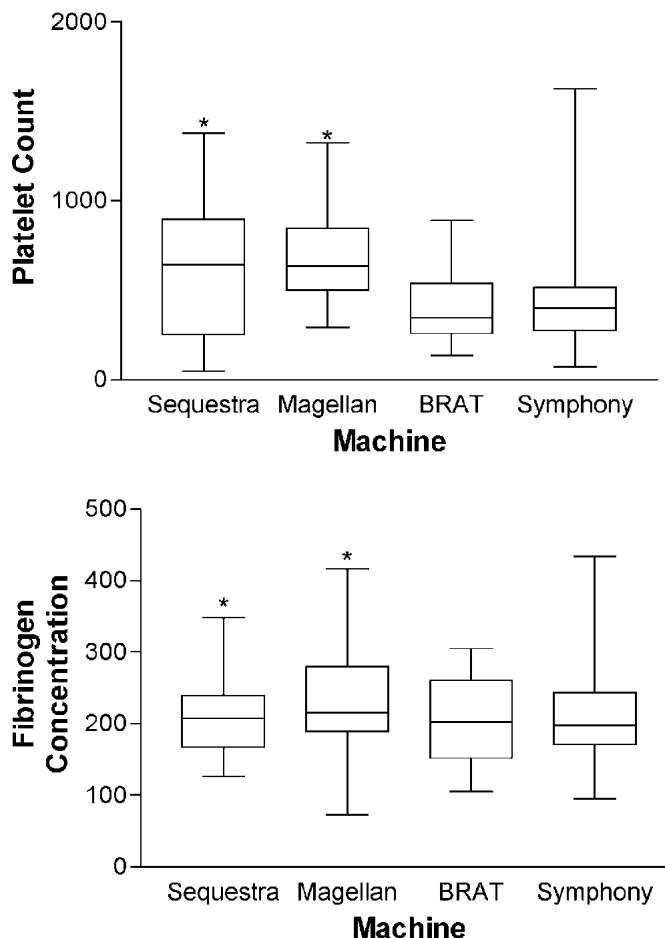


Figure 1. Platelet count variation (upper graph) and fibrinogen concentration (lower graph) in the final PRP are shown by the type of machine used for manufacture. In these box and whisker diagrams, the whiskers represent the range of platelet counts whereas the boxes represent the average and the 25th and 75th percentile. In both analyses, the Sequestra and Magellan had significantly higher results when compared with the other two devices.

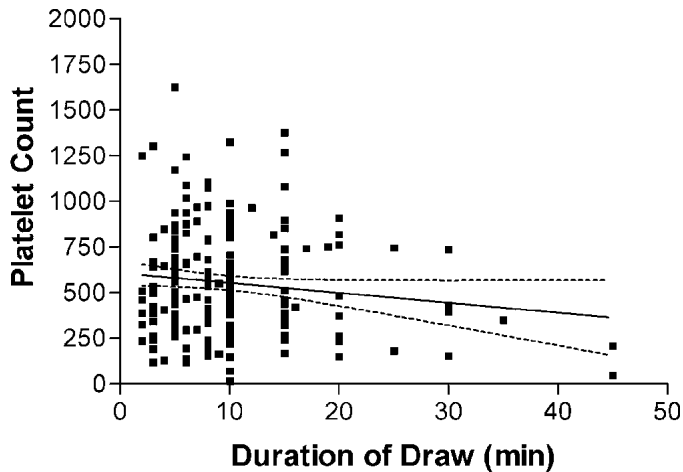


Figure 2. Shown is the relationship between the duration of the blood draw and the platelet count of the PRP. The line represents the linear regression model with its confidence intervals. The slope of the regression line was not significantly different than zero.

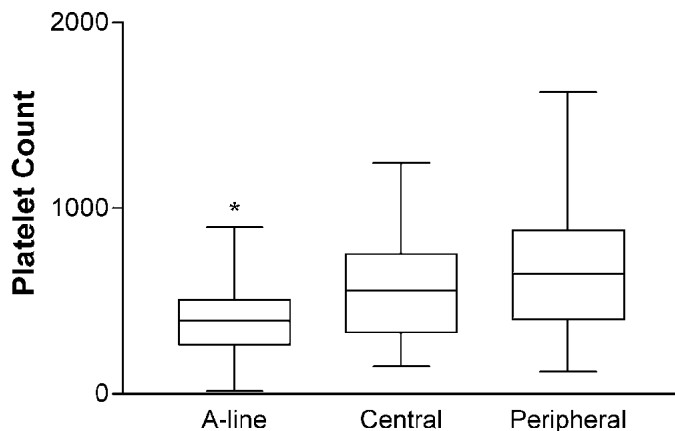


Figure 3. The relationship of the platelet count in the PRP is compared with the method of whole blood withdrawal. It appears that drawing the blood from a peripheral vein is the optimal method. In these box and whisker diagrams, the whiskers represent the range of platelet counts whereas the boxes represent the average and the 25th and 75th percentile. Drawing blood from an arterial line led to significantly lower platelet counts than when blood was drawn from a central or peripheral site.

reveal that patients have not been compliant in discontinuing their antiplatelet medications or herbal supplements. Because many of the patients enrolled in this database were patients undergoing orthopedic surgery where nonsteroidal anti-inflammatory drugs are routinely used for pain relief of the afflicted spine or joint, this lack of gelling may relate to a lack of compliance by the patients in preoperatively discontinuing the use of their nonsteroidal anti-inflammatory drugs.

Many opinions exist regarding an appropriate concentration of platelets in the PRP. From these data, it appears that many factors can influence the platelet concentration. Surprisingly, the method of blood draw suggests that every effort should be made to draw blood from a peripheral

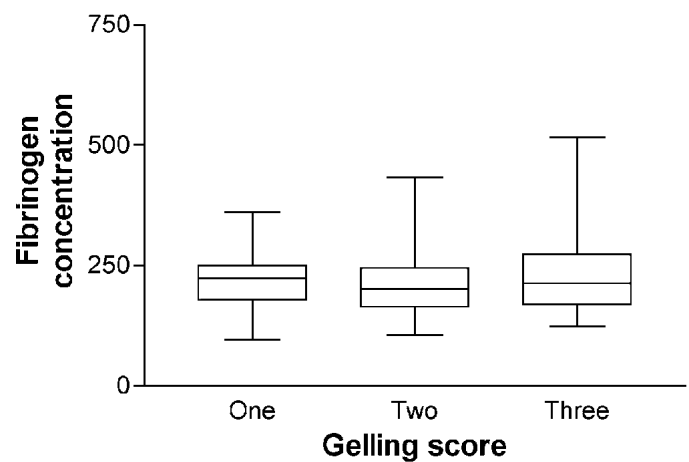
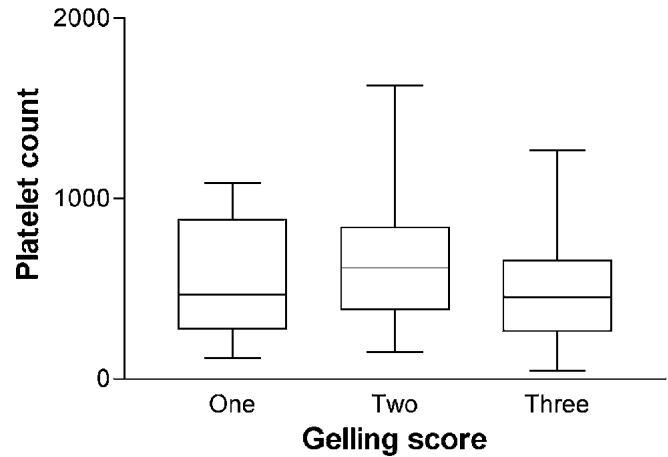


Figure 4. Comparison of gel consistency to platelet count and fibrinogen level. Gel consistency was graded by the surgeon (soupy = 1, firm = 3). The line represents the linear regression model with its confidence intervals. The slope of the regression line was not significantly different than zero for either parameter.

vein. Unfortunately, this is not always feasible for a number of reasons. These reasons primarily relate to limited manpower to draw the blood in a preoperative setting and a desire to not interfere with the progress of the surgical procedure. Our service generally performs the venipuncture after anesthetic induction and prior to surgical incision during the period of time when the patient is being positioned and prepped. From our experience, we have found that drawing a unit of blood from a peripheral vein is difficult while a patient is anesthetized. As to why this is the case is difficult to understand in light of the venodilation that routinely occurs in response to inhalation anesthetics.

The platelet concentration in PRP manufactured by the Symphony system warrants added discussion. From the data presented in this analysis, it appears that the Symphony system produces the least concentrated PRP product. Several points of caution need to be made before drawing this conclusion. First, in most of the cases where

the Symphony system was used, blood was drawn through an arterial line instead of using the manufacturer supplied 16-gauge butterfly needle. In addition, negative pressure was applied via syringe aspiration rather than allowing the blood to flow by gravity. Increases in shear force applied to the platelets resulting in platelet aggregation may result by aspirating blood through a 20-gauge arterial catheter. As is seen in Figure 3, the method of blood draw can significantly influence the PRP platelet count. Thus, lower platelet counts may have resulted from higher shear stress and platelet aggregation as a result of the method of blood draw. Another factor that may influence the final platelet concentration in PRP manufactured by the Symphony system is the amount of plasma that is used to reconstitute the platelet plug. In the Symphony system, the centrifugation process will result in a plug of platelets at the bottom of the disposable cup. Plasma is removed to a fixed volume and the remaining plasma is used to reconstitute the platelet plug. By varying the plasma volume used to reconstitute the platelet plug, varying platelet concentrations can be achieved. This suggests an interesting question for future research. There is a need to determine whether the proposed outcome benefits relate to platelet concentration or whether they relate to total platelet numbers. The last point of interest regarding the Symphony system is that the Symphony system used was a first generation device. Subsequent improvements in the technology may have improved the PRP product manufactured.

As with the manufacture of any blood product, platelet gel should have quality standards applied to its production. The data from this analysis would suggest that wide variation exists in the PRP product depending upon many factors. It could be assumed that the subsequent growth factors from these products would also have great variation. For these reasons, an understanding is needed of the necessary factors to achieve the claimed outcome benefits. These standards could incorporate measures of platelet concentration, fibrinogen concentration, leukocyte and red cell contamination, or gelling capability. The ultimate goal of platelet gel is to enhance wound growth through platelet derived growth factors. Thus, measurement of growth factors would be the best quality check of this product. Unfortunately, research into the outcome enhancement that platelet gel may offer is lacking. Because of this lack of data, the specific growth factors or the mi-

lieu of growth factors necessary to achieve outcome benefit is unknown. This lack of knowledge combined with the significant cost of doing growth factor assays on a routine basis make growth factor assays as a standard quality measure unrealistic. Thus, future research should be directed at indirect ways of assessing the quality of the PRP and of the platelet's ability to contribute growth factors to the wound.

In conclusion, no correlation was found between the patients' platelet count and that of the resulting PRP. As has been described in this database analysis, there are many steps in the process of producing the platelet gel that can result in variation of the final product. In future studies, great care should be made in assuring that the PRP product is manufactured in a consistent, repeatable fashion.

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