Concepts About Current Conditions for the Preparation and Storage of Platelets

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PLATELETS have been transfused since the mid- to late 1960s after it was shown that they promoted hemostasis in thrombocytopenic patients and could be efficiently separated using plastic container systems. Initially, platelets were prepared and stored at 1 to 6°C as platelet-rich plasma prior to transfusion. The observation that platelet-rich plasma could be recentrifuged to form a platelet concentrate has initiated 2 decades of research that have led to the current conditions for preparation and storage.

The changes in Food and Drug Administration platelet storage regulations in the last 15 years provide an overview of the results of research conducted over the last 20 years. Initially, in 1974, it was set forth that platelets could be stored for up to 3 days at 1 to 6°C or 20 to 24°C. With the introduction of more gas-permeable containers and more studies showing detrimental effects of cold storage, the Food and Drug Administration, in 1982, extended the storage time at 20 to 24°C to 5 days and reduced the maximum storage period at 1 to 6°C to 2 days. The storage time for 20 to 24°C stored platelets was extended to 7 days in 1985 and reduced back to 5 days in 1987 due to concern about the potential for bacterial growth with extended storage.

This review reports on the background for practices being used currently to prepare and store platelets and discusses the rationale for the implemented changes in the standards of maximum storage time. Conditions for storage have been based primarily on data obtained using platelets prepared from units of whole blood. The applicability to the storage of platelets produced by apheresis technology will also be addressed. In addition, there is a consideration of various experimental procedures, which may have the potential of improving the retention of platelet properties during storage. Also, a section on tests that can be used to routinely monitor the quality of platelet suspensions is included.

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INFLUENCE OF PREPARATION CONDITIONS ON RETENTION OF PLATELET PROPERTIES DURING STORAGE

Conditions under which platelet concentrates are prepared from units of whole blood (Table 1), besides influencing platelet yield and the residual level of erythrocytes and leukocytes levels, can affect the retention of platelet properties believed to reflect platelet viability and function during storage. Although current conditions have been in use for many years, recent data, albeit limited, indicate that some parameters may not be fully optimal. A survey of conditions currently in use, and a discussion of recently published studies is included in this review to provide a more complete description of the controllable factors influencing the quality of platelet concentrates. As platelet suspensions are predominantly being prepared from platelet-rich plasma, conditions associated with this approach will be emphasized. The potential benefits of using buffy coats will also be addressed.

Anticoagulant-Preservative Solutions

Blood that is to be used to prepare platelets must be collected into a solution containing citrate as the anticoagulant. Ethylenediamine tetraacetate (EDTA) cannot be used because it causes platelets to lose their normal discoid shape and to become spherical, a change associated with a reduction in viability.[1] Heparin cannot be used because it activates platelets resulting in clumping.[2] In addition, EDTA and heparin can cause adverse in vivo reactions.

The preservative components of the solution that are used for red cell storage, phosphate, dextrose and adenine, do not seem to adversely influence platelet properties based on comparative studies with various anticoagulant preservative solutions (ACD, CPD, CPDA-1, CP2D) that have been used over the last 20 years.[3] Glucose is needed as a metabolic energy source. However, it is possible that the plasma itself may provide sufficient glucose for 5-day storage.

Table 1. Current Conditions for Preparation of Platelets

Citrated whole blood
Two-step centrifugation procedure
Rest period before resuspension of platelet pellet

Although citrated plasma is the preferred storage medium at this time, it is apparently not optimal as processes associated with undesirable platelet alterations such as thrombin formation, complement activation,[4] and release of beta-thromboglobulin(s) occur in such a medium. As will be discussed in a later section, approaches are currently being explored to improve the medium for storage.

Centrifugation Conditions

A variety of centrifugation conditions have been used to prepare platelet-rich plasma (PRP). Overall, they can be grouped into three categories: approximately 1,000*g* for 6 to 9 minutes, approximately 2,000*g* for 5 minutes, and approximately 2,600*g* for 3 minutes. (Each time refers to the time setting and does not include braking time.) Satisfactory platelet yields (mean of at least 7×10^{10}) can be achieved using conditions that fit each of these categories. Until recently, it was assumed, in view of the lack of specific data, that PRP preparation conditions did not influence the retention of important platelet properties during storage. Recently, a report indicated that the extent of platelet activation, increase in the pH level, and lactate dehydrogenase discharge, (an indicator of membrane damage) was enhanced when whole blood units were centrifuged at 2,000*g* for 5 minutes as opposed to 2,600*g* for 3 minutes.[5] These data indicate that the issue of centrifugation conditions for PRP preparation may need further investigation.

Platelet-rich plasma is produced, if need be, immediately after phlebotomy, as it is assumed in view of no relevant data to the contrary, that a holding period prior to centrifugation is not required. Recently, results of a study were published which indicate that platelet activation during storage of concentrates is reduced if units of whole blood are held for a few hours before centrifugation to allow the platelets to become deactivated.[6] The data suggest that this issue also needs further exploration.

The concentrating of platelets in platelet-rich plasma has long been thought to induce deleterious changes as a result of the packing of the platelets against each other and the container walls. Release of alpha granular proteins during preparation and storage[7] has served as the basis for this concept. A recent study comparing the properties of platelets prepared from platelet-rich plasma and alternatively using buffy coat that was processed with a soft spin harvesting step ($170 \times g$ for 5 minutes) indicated a striking benefit with the use of the buffy coat fraction. Platelet suspensions prepared in this manner exhibited a greater degree of aggregation and ATP secretion 2 hours after preparation with better retention of other properties including extent of shape change and reversal to osmotic shock during a 4-day storage period at 20 to 24°C.[8]

Changes induced as a result of the concentrating step may also reflect excessive centrifugal force.[9] Although comparative data are not available, it appears that centrifugation at about 3,000*g* for 6 to 8 minutes can be considered as preferred based on the viability data that has been generated documenting that platelets can be satisfactorily stored for 5 to 7 days.

Resuspension of Platelet Pellet

Resuspension of the platelet pellet is performed after a resting period at 20°C to 24°C. Such a procedure is necessary to prevent formation of aggregates from apparently activated platelets. The time for the resting period is routinely between 1 and 2 hours, although the optimal period is unclear because of the lack of comparative data.

Resuspension, per se, can be accomplished by kneading or using storage agitators. Here again, although comparative data have not been collected, there is no reason to select one procedure over another.

Time Between Phlebotomy and Platelet Concentrate Preparation

Since many whole blood units are collected at sites distant from processing centers, it must be possible to hold them for extended time periods under conditions that prevent their cooling to below 20°C prior to preparing platelets. It has been shown that platelets can be harvested and stored satisfactorily if processing is delayed for as long as 6 or 8 hours.[10] On the other hand, as noted previously, a recent report indicates that there is an enhanced risk of damage through activation during

storage if units of whole blood are processed within 1 hour of phlebotomy.[7]

The Influence of Leukocytes on Platelet Suspensions

Routinely prepared platelet concentrates contain leukocytes. The level is quite variable with mean values between about 5 and 10×10^7 per platelet concentrate. Biological factors, centrifugation protocols, and processing conditions affect the number of residual contaminating leukocytes that are present. The relative influence of the many factors that appear to play a role is not understood. One report indicates that careful centrifuge balancing and subsequent PRP expression (1 cm from the top of the blood bag compared with stopping expression when the red cell interface reaches the top of the bag) reduce leukocyte levels.[11]

In the late 1970s and early 1980s, there was a controversy regarding whether the leukocyte production of lactic acid caused or enhanced the decrease in the pH level during platelet storage in first generation containers.[12] The use of more oxygen-permeable containers in which a marked decrease in the pH level is rarely seen alleviated such concern.

Currently, as the presence of leukocytes have the potential of causing febrile reactions, immunization, and transmittal of viruses, there is a growing interest in preparing leuko-poor platelets. Procedures involving filtration or centrifugation in a special container reduce the leukocyte level by between one and three logs. Leukocyte removal, per se, does not have a detrimental affect on platelet properties, when assessed after processing.[13,14] However, in vitro and in vivo data on leuko-poor platelet suspensions stored for up to 5 days at 20 to 24°C still needs to be collected. This issue will no doubt receive extensive attention during the coming years as regional processing centers prepare more leuko-poor platelet products. Recently, a sterile connecting device to attach a filter has become available. This will allow for the storage of leuko-poor platelets.

Use of Buffy Coats to Prepare Platelet Concentrates

Although platelet-rich plasma is the predominant starting component for preparing platelet suspensions for transfusion, buffy coats can also be used.[15] With this approach, harvested buffy coats are centrifuged at a relatively slow speed, which concentrates the platelets into an expressible plasma supernatant phase. For a number of years, it has been thought that use of the buffy coat, which excludes the need to form a platelet pellet, reduces the extent of undesirable activation. Recently, comparative studies have documented that the buffy coat approach causes substantially less storage-associated activation.[8]

STORAGE PARAMETERS

The current storage conditions for platelet concentrates (Table 2) were developed subsequent to determining that platelet concentrates could be easily prepared. The two major issues have been storage temperature and plastic container properties. Until recently, the contents of the preserving medium have received only limited attention.

Temperature for Storage

Initially, platelets were stored at 1 to 6°C because it was known that red blood cells could be satisfactorily preserved at this temperature range. It was reported in 1969—when the value of transfusing platelets was emerging—that storage at cold temperatures markedly reduces posttransfusion viability, evaluated as percentage recovery and survival time.[16] In addition, these in vivo viability studies in which platelet-rich plasma units were stored for 24 hours at various temperatures between 4 and 37°C indicated that 22°C (now taken as 20 to 24°C) best maintained the properties of platelets. These studies were followed by a period of time in which the benefits of cold storage (1 to 6°C) versus the benefits of room temperature storage (20 to 24°C) were subjected to extensive experimentation, and even controversy. The major reasons for continued storage at 1 to 6°C in the early 1970s, after procedures to store platelets as concentrates were developed, were concern about the potential for bacterial proliferation during room temperature storage and the simplicity of storage

Table 2. Current Conditions for Platelet Storage

20-24°C temperature range
Second-generation containers
45-60 mL volume
Continuous agitation
Need for glucose in anticoagulant preservative solution

conditions (no need for incubators and agitators). In addition, comparative studies showed better aggregation with cold stored platelets.[17] Also, platelets stored at 20 to 24°C showed a marked decrease in the pH level and the accompanying loss of viability at high platelet counts during storage.[18] In part, this reflected inappropriate conditions such as storage without agitation, suboptimal plasma volumes, and use of first-generation containers made from polyvinylchloride plastic plasticized with diethylhexylphthalate, which have limited gas permeability. These issues will be discussed in more detail below.

Data collected in the early and mid 1970s substantiated the concept that 20 to 24°C was the preferred temperature range.[19,20] Safety was attested to in a number of studies that showed that the great majority of platelet concentrates stored at 20 to 24°C for 3 days did not contain bacteria on culturing.[3] The superior prolonged effectiveness after transfusion of platelets stored for 3 days assessed by corrected count increments and bleeding time measurements, as shown in two separate studies,[19,20] complemented the better retention of viability and appears to be the main reason for the nearly universal acceptance of 20 to 24°C as the current storage temperature range.

More recent data have confirmed that 20 to 24°C is the optimal range considering viability/function issues. The appropriateness of 20°C as the lower limit of the room temperature range was confirmed in an in vivo viability study that showed that storage at 18.0°C and 19.5°C reduced mean life spans compared with that observed at a storage temperature of 21°C.[21] Also, the retention of the ability of platelets stored at 20 to 24°C to aggregate has been shown in many studies. The original concept that the aggregation potential was lost reflected the use of one activating agent at a time. Aggregation has been observed, probably more appropriate to the in vivo situation, in the presence of two activating agents (synergistic aggregation).[22,23] Although there are only limited data assessing retention of platelet properties at storage temperatures above 24°C, increased lactate formation at such temperatures will no doubt be detrimental.

A delay in achieving full hemostatic effectiveness of room temperature stored platelets on transfusion, as attested to by bleeding time measurements, was an issue of concern in the 1970s even though there was limited data documenting such a phenomenon.[24] This issue has been abrogated in recent years because of the substantial use of platelets for prophylactic purposes. In addition, there are no recent reports regarding detrimental effects when room temperature–stored platelets are transfused to patients who are actively bleeding.

There are data showing that platelets can be stored in a citrated-plasma medium for up to 7 days at 20 to 24°C with similar posttransfusion corrected-count increments, and comparable percentage recoveries and survival times[25-27] as 3- or 5-day-stored platelets. The reduction in the storage time to 5 days in 1986, a year after a 7-day dating period had been approved in the United States, reflected concern about the potential for bacteria to proliferate during extended storage at 20 to 24°C based on data collected in a few studies.[3]

Conditions to Maintain pH During Storage

With conversion to the 20 to 24°C storage temperature, it was shown that many platelet concentrates stored for the maximum time of 3 days, with plasma volumes of approximately 50 mL and agitation, exhibited markedly reduced pH levels. In up to 25% of the concentrates, the pH (for this review, pH levels refer to room temperature levels) was below 6.0 to 6.2,[18,28] an event that was associated with low posttransfusion autologous viability.[29] This reflected high levels of lactic acid and carbon dioxide accumulation in the storage containers caused by limited gas permeability of the containers. This led to the development of new containers (second generation) with improved gas transport, which with the use of appropriate conditions such as agitation, has allowed for the retention of pH levels near 7.0 after 5 to 7 days of storage. As a result, posttransfusion viability properties are retained at satisfactory levels for up to 7 days. The three conditions that influence the retention of pH levels, container size and volume, plasma volume, and agitation, will be considered individually and collectively to describe how they influence the optimal maintenance of the pH level.

Containers for storage. The plastic container used to store platelet concentrates is the principal factor responsible for maintaining or not maintaining platelet concentrate pH levels near 7.0 (between approximately 6.8 and 7.4) during storage. The polyvinylchloride plastic (with diethylhexylphthalate as the plasticizer) that was associated

with the pH reduction problem was suboptimal because of its limited permeability toward oxygen and to a lesser extent toward carbon dioxide. This concept emerged when it became known that the pH reduction was associated with increased lactic acid production and low plasma oxygen levels and was influenced to a large degree by the platelet count per unit volume.[18] It recognized that platelets during storage at 20 to 24°C carry out energy metabolism through glycolysis and oxidative phosphorylation and that metabolism at insufficient oxygen levels is channeled preferentially through the lactic acid-producing glycolytic pathway. Insufficient oxygen levels inside the containers were observed mainly when platelet counts were high.

The additional need to have the plastic permit the escape of metabolically produced carbon dioxide from the container relates to the fact that glycolytically derived lactic acid is converted to sodium lactate and carbonic acid on its reaction with bicarbonate, the main buffer of plasma. Once bicarbonate is exhausted, which occurs at lactic acid levels of 20 to 25 mmol/L, a rapid decrease in the pH level occurs. Also there is a pH lowering effect of carbonic acid, which can be reduced by allowing its dissociation product, carbon dioxide, to efflux through the plastic.

Understanding the reasons for the pH reduction has led to the development of so-called second-generation containers with increased and apparent near optimal permeability toward oxygen and carbon dioxide. Three types of permeable containers are now available.[30-32] One uses a blend of polyolefin plastic constituents without plasticizer, and a second type uses polyvinylchloride plastic but with a trimellitate plasticizer. A third type also uses polyvinylchloride plastic with diethylhexylphthalate plasticizer but uses a reduced thickness of the container wall from that of first-generation containers. Each of the three types of containers allow for the maintenance of platelet concentrate pH levels above 6.0 and have been approved for 5-day storage of platelet concentrates.

The polyolefin container with its nominal 300 mL volume provides an adequate surface area for satisfactory gas exchange. The other two containers are constructed with a 400 mL volume; the added surface area helps to provide satisfactory gas exchange.

Oxygen permeability for 4 second generation containers (PL732 (polyolefin), CLX (PVC, trimellitate) PL1240 (PVC, trimellitate), XT-612 (PVC, DEHP reduced thickness)) and the first generation PL146 container is shown in Fig 1. Also shown is the relationship between oxygen diffusion and the range of platelet counts in which oxygen consumption does not exceed oxygen availability. When platelet counts are above these levels, anaerobic metabolism through glycolysis is increased resulting in enhanced lactate formation. The relationship between gas transport, metabolism, and plasma buffering processes are schematically represented in Fig 2.

The oxygen and carbon dioxide levels in platelet concentrates stored in second generation containers with agitation is respectively approximately 100 and 40 mm Hg after 24 hours of storage. First generation concentrates with pH levels near 7.0 and low to medium platelet counts exhibited oxygen and carbon dioxide levels of approximately 20 and 80 mm Hg. These values correspond to platelet counts of approximately 1 to 1.5 $\times$ $10^6/\mu L$.

Volume of platelet concentrate. In the presence of adequate levels of oxygen, the actual pH levels reflect the overall metabolic activity through glycolysis and the balance between lactic acid formation and carbon dioxide efflux. The key parameter affecting metabolic activity is the platelet count and possibly the level of specific platelet subpopulations.[18,28] At high platelet counts, the total amount of lactic acid is increased causing a decrease in the pH level. At low platelet counts,

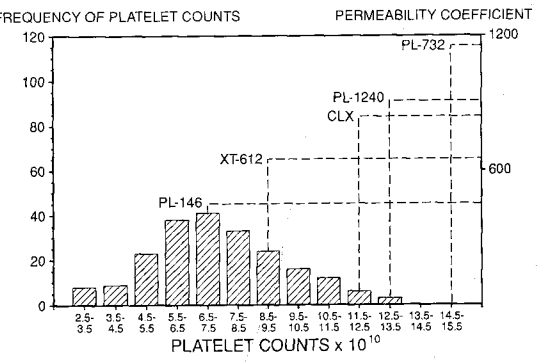

Fig 1. Oxygen permeability of four second-generation platelet storage containers and one first-generation (PL 146) platelet storage container. The horizontal dashes indicate the container oxygen permeability in millimoles per minute per atmosphere of oxygen, and the vertical dashes indicate the maximum range of platelet counts that can be tolerated so that an adequate oxygen level in the platelet concentrate is maintained. Also included is a distribution of platelet counts in a sampling of 215 platelet concentrates. The left ordinate indicates the frequency of the various platelet count ranges.

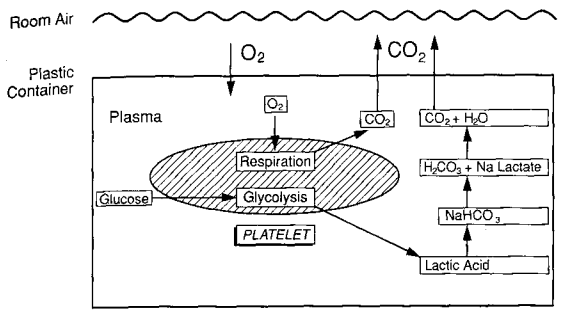

Fig 2. Gas transport, metabolic, and plasma buffering processes that influence pH maintenance during platelet storage.

total lactic acid formation is reduced, causing the pH level to increase because of the efflux of carbon dioxide. Also, since carbon dioxide is produced as a byproduct of respiration causing a decrease in the pH level because of carbonic acid formation, the lower the platelet count the lower the amount of carbon dioxide produced, thus causing less of a fall in the pH level.[33]

The volume of platelet concentrate is a measurable parameter that can modulate a decrease in the pH level. Decreasing the volume increases the platelet count per unit volume and shifts the balance toward a decrease in the pH level, in part because the buffering capacity (total bicarbonate) is reduced. Increasing the volume decreases the platelet count and shifts the balance in favor of carbon dioxide efflux. Routinely, 45 to 60 mL of plasma is being used because such a volume range maintains the pH level of the majority of a representative group of platelet concentrates, with the routinely found wide variability in platelet counts, near 7.0. Thirty milliliters appears to be insufficient to store platelet concentrates in PL 732 containers based on limited data.[30] However, a recent report claims that a 30 mL volume is adequate for platelet concentrates stored in the PL 732 container,[34] indicating that additional studies are needed to better define the minimum allowable volume.

In practice, the pH after 5 days of storage at 20 to 24°C rarely reaches 6.0 to 6.2 except at counts above $2 \times 10^6/\mu L$ (equivalent to above 1.0×10^{11} platelets per 50 mL) when the container is a second generation container made of polyvinylchloride plastic. The pH level increases above approximately 7.4 (measured at room temperature) at counts below $1 \times 10^6/\mu L$ (equivalent to 5.0×10^{10} platelets per 50 mL) especially when using the polyolefin container. If there is one problem with the second generation containers, it relates to the increase in the pH levels at very low platelet counts ($<1 \times 10^6/\mu L$) as high pH levels are associated with reduced autologous viability.[30] With first generation containers, an excessive reduction in viability was detected above the pH level of 7.4.[35] Sufficient data to establish an overall acceptable upper pH level still need to be generated for the second generation containers. It is possible that high pH levels indicate a deleterious effect of storage which may be enhanced at low platelet counts.

Beneficial influence of agitation. Agitation facilitates the transfer of gases across the walls of the plastic containers and is required for achieving sufficient influx of oxygen and efflux of carbon dioxide through second generation containers and hence retention of pH levels. One study showed that the mean pH level of platelets stored for 5 days in polyolefin containers decreased to 6.3 without agitation, while agitated concentrates exhibited levels near 7.0.[36]

The use of continuous agitation is routinely used in the storage of platelet concentrates except during shipment from blood centers or subsequent to issuing by hospital blood banks. Short periods without agitation do not appear to be deleterious. One recent study did not find any subsequent change in vitro platelet properties of concentrates stored in second generation containers following discontinuation of agitation for 24 hours. The concentrates were retained in a cardboard insulated shipping box at 20 to 24°C. The pH levels of concentrates held in polyolefin containers were found to be 0.2 units less than continuously agitated concentrates.[37]

Additional considerations of agitation. It has been speculated that agitation may also be beneficial because it maintains the platelets in a state of suspension over a prolonged period of time and equalizes the buildup of metabolic products throughout a suspension. However, this concept has never been validated. In fact, the satisfactory storage of platelets without agitation by compressing the platelet concentrate into a thin layer that allowed for satisfactory gas exchange argues against the concept.[38]

Agitation can also have a detrimental influence on platelets during storage. Subsequent to documentation that agitation was needed to facilitate gas exchange,[18] it was realized that selected forms of agitation could cause excessive platelet damage

when platelets were stored in specific containers.[35] As a result, interest in evaluating the influence of mode and speed of agitation on the retention of platelet properties during storage was stimulated, especially at the time of the development of the second generation containers. This reflected a desire to delineate optimal agitation conditions with the available equipment.

In vitro and in vivo studies comparing available forms of agitation were conducted with platelets stored in the polyolefin and polyvinylchloride-trimellitate containers.[39] The end-over-end (tumbler), flatbed (to and from), and elliptical forms of agitators were used. One agitator-container combination, involving the polyolefin container and the elliptical agitator (operating at 6 rpm) was shown to cause an excessive reduction in retention of platelet properties during storage. The damaging factor was shown to be the speed since studies with an elliptical agitator (operating at 1 rpm) did not show excessive deleterious changes.

Data delineating reasons why specific agitation conditions induce a situation that causes enhanced platelet damage have not been generated. Possible reasons however have been suggested. These include the possible induction of harmful interactions between platelets and the walls of the container and/or the occurrence of greater than normal shear stress.[35]

Storage time. Identifying the need to store platelets at 20 to 24°C and to use conditions that maintain pH levels near 7.0 provided the basis for extending the storage period beyond 3 days, a standard that was adopted in 1974. The initial extension to 5 days in 1982 was followed by a lengthening of the maximum storage period to 7 days. This was deemed appropriate because 5- and 7-day-old platelets exhibited only a 30% reduction in the in vivo percentage of recovery compared with fresh platelets.[25,26]

The rollback to 5 days in 1986 reflected concern about the potential for bacterial growth during extended storage in second generation containers. It was based on data that showed that bacteria, apparently of skin origin, were detectable in a limited number of platelet suspensions.[40] It recognized that although the risk of transfusing bacteria-containing concentrates was low, as had been previously noted, a 5-day maximum storage period was a preferred condition considering overall issues pertaining to safety and supply of platelets.

Use of current conditions for storage platelets as pools. In the near future, it may be possible to store platelets isolated from whole blood as pools of 4 to 8 platelet concentrates using the principles developed for storage of single units. A recent report indicated that if a sufficiently large container, composed of a second generation gas permeable plastic is used, platelet properties are maintained during a 5-day storage period at 20 to 24°C with agitation. Moreover, the addition of lymphocytes from multiple donors did not generate a mixed lymphocyte reaction.[41]

CONDITIONS FOR THE STORAGE OF APHERESIS PLATELETS

Conditions being used to store apheresis platelets parallel those being used to store platelets isolated from units of whole blood. Their development was based, primarily, on the knowledge gained using whole blood–derived platelets that adequate gas exchange is critical for maintaining platelet viability.

When apheresis platelets were first stored in the mid 1970s, at 20 to 24°C, it became apparent that the 300 mL container was inadequate for storing an average apheresis harvest even with agitation because of inadequate container gas exchange.[42] A rapid decrease in the pH level occurred during a 24-hour-period, which was considered allowable in view of the open nature of the apheresis systems available. It is now known that this reflected production of lactic acid as a result of insufficient oxygen transfer. A 2,000 mL container with enhanced gas transport capacity, also composed of a first generation polyvinylchloride-diethylhexylphthalate plastic, was developed and became routinely used when platelets prepared by apheresis were to be stored for any substantial period of time up to 24 hours at 20 to 24°C. Data that have been collected substantiated that the in vitro platelet properties[42,43] and autologous viability parameters[44] are maintained in this container.

The availability of closed system apheresis devices and second-generation plastics was followed by the development of sterile, disposable software packages containing 5-day storage containers for apheresis procedures.[45,46] Packages in use have two integrally attached 1,000 mL containers composed of either polyolefin, polyvinylchloride-trimellitate, or a polyvinylchloride plastic, which uses a fairly new citrate-based plasticizer termed

Citroflex.[47] (The potential use of this plasticizer for containers to be used to store platelets derived from units of whole blood is under evaluation.[48])

Routinely, a plateletpheresis harvest—usually 3 to 6×10^{11} platelets in 200 to 300 mL plasma—is subdivided equally into the two containers. The need for two containers reflects the fact that due to limited gas transport capacity, each of the containers can be used to store only up to about 3.5×10^{11} platelets with retention of adequate pH levels during 5-day storage period.

Apheresis platelet units are agitated during storage to provide adequate gas exchange in a similar way as for platelets separated from units of whole blood. Studies have not been conducted to evaluate how speed and mode of agitation influences the retention of the properties of platelets harvested by apheresis technology, but there is no reason to believe that the data would be any different from that obtained with platelets that are prepared from whole blood.

Apheresis platelets are collected and stored in either ACD-A or ACD-B as compared with CPD, CPDA-1 or CP2D, which are used to collect whole blood units. Although these anticoagulant-preservative solutions allow for the retention of platelet properties at satisfactory levels, it is quite possible that more customized solutions will better retain relevant properties as may be the case for platelets isolated from units of whole blood.

POTENTIAL APPROACHES THAT MAY IMPROVE CURRENT PROCEDURES

Development of Specific Storage Media

Although the conditions that are in current use prevent certain changes while minimizing others, storage of platelets is still associated with substantial alterations in quality.[24] These changes appear to have a biochemical basis, which indicates that they could be minimized by altering the suspension media for platelet storage. The current media reflect what is being used to store red cells and may indeed be suboptimal for platelets.

The issue of suspension media has received limited attention until recently. However, the use of a synthetic medium for resuspension and storage of platelets may afford a medium that improves the quality of stored platelets, while also offering the opportunity for saving more plasma to be used in fractionation and in the treatment of patients. There are also other potential advantages in using a plasma-free medium: transfusion reactions caused by noncompatible plasma proteins may be avoided and procedures to inactivate residual viruses that may be present may be carried out more easily in a nonplasma medium.

At present, there are no commercially available synthetic media for resuspension and storage of platelet concentrates. A serious problem in the development of a practical and safe preservation medium is the need to compensate for the substantial generation of lactic acid by platelets. During storage in a synthetic medium at 20 to 24°C, there will be a resulting decrease in the pH level unless a buffer system is added. As noted previously, a decrease in pH to levels below 6.2 has been related to substantial loss of platelet viability.[24]

However, it has been difficult to develop a practical and safe solution with high buffering capacity that does not pose manufacturing problems. Part of this problem is that with a glucose-containing solution having a pH level of between 6.8 and 7.0, which is necessary for storage of platelets, heat sterilization cannot be carried out due to caramelization of gluose. This means that the buffer solution has to be sterilized and stored separately from the remaining medium, with the combining of these two solutions at the time when the platelet concentrate is prepared.

In view of these issues, the possibility of storing platelets in a glucose-free medium is being explored. It has been shown that platelets derive 85% of their energy from respiration and that glucose is not the major energy substrate in respiration, indicating that glucose may not be essential in platelet storage.[33] However, more recently, it has been suggested some glucose may be needed for platelet storage.[49] In these studies, platelets were prepared in a manner that resulted in less than a 1% CPD-plasma carry-over to the synthetic medium which meant that less than 0.5 mmol/L glucose was present in the final suspending medium. The studies showed that although some platelet viability remained after 5 days of storage in the glucose-free medium, it was significantly reduced as compared with storage in the same medium containing 35 mmol/L of glucose.

Other laboratories have been more successful in using glucose-free additive solutions. However, due to different preparation technqiues, substantial amounts of plasma carry-over (20% to 40%) were

present in the final storage media resulting in glucose levels higher than 3.2 mmol/L. Under these conditions, Adams and Rock[50] have found that platelets stored in Plasmalyte A (Baxter Healthcare, Deerfield, IL), which is a commercially available glucose free crystalloid solution, have good in vitro characteristics after 5 days of storage.

Satisfactory posttransfusion viability with platelets stored in Plasmalyte A, as shown by count increments studies in thrombocytopenic patients, have recently been documented.[51] Apparently, the amount of glucose metabolized by the platelets during a 5-day storage period in this medium was sufficient for normal energy metabolism.[52] Studies have shown that the amounts of glucose consumed and lactate produced over a 5-day period in Plasmalyte A are the same with or without added glucose.[52] Interestingly, the amount of lactic acid produced was only 50% of what is observed with 5 days of storage in CPD-plasma. This explains why no decrease in the pH level was observed since Plasmalyte A does not have a buffer system.

Murphy et al[53] have developed a glucose-free medium containing 25 mmol/L phosphate as a buffer to neutralize lactic acid produced from glucose present in carry-over plasma. Unpaired preliminary studies suggested that platelets stored in this medium for up to 10 days have similar posttransfusion survivals as those stored in plasma. A recent confirmatory study, however, in which paired studies were conducted, demonstrated poorer in vivo posttransfusion results in this medium as compared with CPD-plasma.[54]

A synthetic medium containing glucose with bicarbonate as the buffering system has been developed by Holme et al.[55] Extensive testing has suggested that platelet in vitro and in vivo characteristics are better maintained in this medium compared with storage in CPD-plasma. This medium has the added advantages of allowing for maximal plasma removal, while it may also be used for storage of red cells. Recent studies have also shown that a slight modification of this medium with addition of adenine allowed for satisfactory posttransfusion recoveries of 42-day-old stored red cells.[56] However, a drawback with this glucose-containing medium is that the sodium bicarbonate has to be sterilized and stored in a separate container, thus increasing manufacturing costs.

Modification of Plasma Medium

A different medium modification approach having the goal of improving retention of platelet properties during storage has involved adding cyclic adenosine monoplasplate-enhancing substances and inhibitors of proteolytic proteins to the anticoagulant solution. The most promising formulation appeared to include prostaglandin E_1; theophylline; aprotinin, an inhibitor of plasmin and kallikrein; and an inhibitor of thrombin, such as hirudin.[2] This approach is being pursued to inhibit biochemical changes that apparently reduce the quality of platelets when using the current storage techniques.

ROUTINE QUALITY CONTROL OF STORED PLATELET SUSPENSIONS

Although it is beyond the scope of this report to delineate the various measures that can be used in a research setting to assess in vitro and in vivo retention of platelet viability and function parameters, it is worthwhile to note that there are selected parameters that can be routinely assessed to assure satisfactory preparation and storage (Table 3). Procedures to assess the quality of a platelet suspension include enumeration of platelet numbers using hemocytometry or an automated cell counter, measurement of plasma pH at room temperature or 37°C, evaluation of suspension volume, and visual inspection to assure that the suspension does not have an excessive number of red cells (red color) and particulate matter and that the swirling (shimmering) properties are maintained.

Standards for adequate number of platelets have been established by the American Association of Blood Banks.[57] For platelet concentrates prepared from units of whole blood, 75% of tested suspensions are required to have at least 5.5×10^{10} plate-

Table 3. Characteristics of a "Good Quality" Platelet Suspension

Platelet count of at least 5.5×10^{10} when using whole blood and at least 3×10^{11} when using apheresis technology
pH level between 6.8 and 7.4 (measured at room temperature)
No excessive amount of red cells and/or particulate matter
Presence of swirling (shimmering) to indicate satisfactory retention of discoid morphology

lets, and for apheresis platelet units, 75% of tested suspensions are required to have at least 3×10^{11} platelets. These levels should be taken as minimum standards as current practices should allow for a mean level of approximately 7.5×10^{10} platelets in 5-day-old platelet concentrates that have been prepared from whole blood and a mean level of approximately 3.5×10^{11} for apheresis platelets.

The pH levels of all platelet suspensions should be between 6.8 and 7.4. The lower pH guideline is substantially above the current 6.0 standard. It reflects what can be achieved routinely with second-generation containers and has as its basis data that shows that there is an accelerated reduction in overall quality at pH levels well above 6.0.[24,58] Although these are continuing questions about a suitable high pH limit, a value of 7.4 is considered as appropriate in view of data which indicates reduced quality at pH levels of 7.5 to 7.6.[30,35] The volume of a platelet suspension needs to be within a specific range to increase the likelihood that the pH level will be maintained in the 6.8 to 7.4 range during storage. The optimal range for platelet concentrates prepared from units of whole blood, with second-generation containers appears to be 45 to 60 mL. Volumes near and above 60 mL can cause pH levels to rise above 7.4, especially when the platelet harvest is around 5.5×10^{10} (or lower). Little is known about the lower limit for the volume, and in view of limited data in the literature, this issue needs further study.

Visual inspection that can be performed without compromising the integrity of the container can be used to ensure that the color of the plasma is straw or slightly pink indicating that there is not an excessive level of red cells. This type of evaluation can also be used to determine that there is not an excessive amount of particulate matter. In addition, holding a platelet-concentrate container near a light source and giving it a flip or a twist allows an assessment to be made about the presence of a swirling (shimmering) phenomenon. This visually detected light scattering has been associated with the retention of the discoid platelet morphology.[59,60] It is known to be absent in room-temperature–stored platelets having pH levels of around 6.2 and below and in suspensions stored at 1 to 6°C. Although the swirling is basically a qualitative measure, it is possible to establish an arbitrary scoring system for assessing the magnitude of the phenomena.[59,60]

SUMMARY

Substantial experimentation over the last 20 years has led to the conditions that are currently used to prepare and store platelets. Although platelet rich plasma is used in most instances to prepare platelet concentrates, there may be some benefit associated with the use of buffy coats as the source component.

Extension of the maximum allowable storage time for platelets to 5 days has been possible as a result of defining the conditions which allow for the better retention of platelet properties. Storage temperature, permeability of the storage container, volume of platelet suspension, and the need to agitate platelets have been identified as key parameters that maintain platelet viability and functional properties. Storage in the 20 to 24°C range prevents a reduction in posttransfusion viability that occurs when platelets are maintained at lower temperatures. Adequate influx of oxygen through container walls to support platelet metabolism and, to a lesser degree, adequate efflux of produced carbon dioxide are essential for maintaining pH levels, a key parameter that also influences posttransfusion viability. Permeability is influenced by container size and material, by use of a satisfactory volume of plasma and by agitating the container. Although platelet concentrates prepared from whole blood have been primarily used to delineate appropriate storage conditions, they also apply to platelets harvested by aphersis technology.

Storage under currently used conditions, although providing products with acceptable clinical efficacy, is associated with a reduction in viability and functional characteristics. The development of storage media, specific for platelets, may minimize the occurrence of deleterious changes.

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