Comprehensive review of platelet storage methods for use in the treatment of active hemorrhage

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This review considers the various methods currently in use, or under investigation, for the storage of platelets intended for use in the treatment of active hemorrhage. The current standard practice of storing platelets at room temperature (RT) (20°C-24°C) optimizes circulating time, but at the expense of hemostatic function and logistical considerations. A number of alternatives are under investigation. Novel storage media additives appear to attenuate the deleterious changes that affect RT stored platelets. Cold storage was originally abandoned due to the poor circulating time of platelets stored at 4°C, but such platelets may actually be more hemostatically effective, with a number of other advantages, compared to RT stored platelets. Periodically re-warming cold stored platelets (temperature cycling, TC) may combine the hemostatic efficacy of cold stored platelets with the longer circulating times of RT storage. Alternatives to liquid storage include cryopreservation (freezing) or lyophilization (freeze-drying). The former has had some limited clinical use and larger clinical trials are underway, while the latter is still in the preclinical stage with promising in vitro and in vivo results. The importance of platelet transfusion in the management of active hemorrhage is now well accepted, so it is timely that platelet storage methods are reviewed with consideration of not only their circulating time, but also their hemostatic efficacy.

INTRODUCTION

While there is ongoing controversy around the ideal ratio of platelets to other blood products, the early transfusion of platelets as part of a massive transfusion protocol has become established practice in the management of acute hemorrhage, particularly in the resuscitation of trauma patients with major bleeding.[1-6] However, current standard platelet storage conditions were primarily developed for the oncological and hematological patient, who is most often transfused prophylactically in the setting of thrombocytopenia. In this case, the most important consideration is platelet-circulating time to reduce the frequency of transfusions. In the actively bleeding patient, the most important consideration is hemostatic function, and circulating time is relatively unimportant as platelets are removed rapidly from the circulation as blood clots are formed. Current practice is to store platelets at room temperature (RT) (20°C-24°C), which maximizes circulating time, but this comes at the expense of properties that are important for treating active hemorrhage.[7] First, RT stored platelets have impaired hemostatic function.[8,9] They also have the highest rate of bacterial contamination of all blood products due to the rapid growth of bacteria at 20°C-24°C.[10] Aside from increasing the risk of transfusion related sepsis, particularly for hemorrhaging patients who typically receive multiple units, the risk of bacterial

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contamination restricts RT storage duration to 5 or 7 days (country dependent).[10] This relatively short storage duration has logistical consequences for maintaining adequate platelet stocks, and may even be prohibitive for maintaining a platelet inventory at all for rural, military, and austere facilities. In addition, RT platelets require near-constant agitation to maintain gas exchange and aerobic metabolism, which adds additional complexity to storing and transporting platelet units.[11] In light of these issues, attention has turned to not only optimizing RT storage, but to investigating alternatives to RT storage altogether.

DETERMINING HEMOSTATIC EFFICACY

Before clinical trials, new platelet storage methods are assessed using a combination of in vitro, ex vivo, and in vivo studies using animal models or human volunteers.[12-16] However, there is a poor understanding of how these tests correlate to recovery, survival, and hemostatic efficacy when the platelets are transfused into thrombocytopenic or actively bleeding patients.[12,14,16] Furthermore, storage-associated defects identified in vitro may be reversible on transfusion.[14,17] Some in vitro tests are validated for recovery and survival, but not necessarily hemostatic function. For instance, a poor hypotonic shock response (HSR) is associated with poor recovery of transfused platelets in vivo,[18,19] as is a low pH and high lactate levels,[12,14] but little is known about any association of these markers with hemostatic function. Whereas a high percentage of disc-to-sphere shape change is associated with reduced circulation time in vivo,[13] but as discussed in detail in this review, spherical platelets are not necessarily associated with a reduction in hemostatic function.

Another commonly used in vitro marker is platelet surface receptor expression, measured using flow cytometry.[15,16] The most commonly measured receptors are glycoprotein (GP) Ib, GPIIb-IIIa, P-selectin, and phosphatidylserine. Expression of these receptors changes during storage, in part due to platelet activation.[20] However, it is still unclear how the changes in receptor expression affect platelet survival and hemostatic function.[14,15] It is likely that a certain degree of pre-activation and up regulation of receptor expression enhances hemostatic function and the evidence for this will be explored in this review.

There are multiple methods available for measuring the platelet functions of adhesion, aggregation, thrombin generation, and thrombus formation in vitro.[12-16] Aggregation response is the most commonly measured, but this does not correlate with platelet recovery and survival after transfusion.[12] There are little data on how aggregation measured in vitro correlates to hemostatic effectiveness in vivo. However, it has been shown that in vitro aggregability correlates moderately well with aggregability scores in an ex vivo transfusion model,[17] and, clinically, impedance aggregometry correlates strongly with bleeding and transfusion requirements following cardiac surgery.[21,22] This suggests that these in vitro tests of function may be a useful marker of hemostatic efficacy but not of recovery and survival, although further validation of these methods is required.

Posttransfusion hemostatic efficacy can be assessed in human volunteers by measuring bleeding time, which is the time it takes for a standardized superficial cut to stop bleeding.[12,16] While this is a direct assessment of in vivo hemostatic function, there are many variables that affect the result, so it is unsurprising that the test is not sensitive or specific for platelet function and is seldom now used.[12,16] Alternatively, animal models provide controlled, standardized conditions for testing hemostatic efficacy in vivo, but with some limitations due to interspecies differences. The efficacy of different platelet products at controlling active hemorrhage has been tested in a variety of animal models.[12]

While many of the tests used to evaluate platelet function have not been validated to correlate with hemostatic function posttransfusion, inferences can still be made based on an understanding of platelet structure and physiology. For example, the number of platelet alpha-granules (comprising 6%-15% platelet volume, and containing adhesive proteins, various inflammatory mediators, growth factors, and immunoglobulins, responsible for much of hemostatic function) falls with prolonged storage, although not as rapidly as the fall in dense granules (containing activating mediators such as serotonin and histamine).[23] Furthermore, the emerging field of systems biology, particularly the subfields of proteomics and metabolomics may in the future identify useful biomarkers of platelet storage quality, as well as provide new insights into the mechanism of the platelet storage lesion, and ultimately lead to improved storage methods.[24-26]

LIQUID STORED PLATELETS

Room temperature

During storage, platelets undergo a collection of structural and functional changes that are referred to as the platelet storage lesion.[27] These include a reversible disc-to-sphere shape change or a breakdown to fragmented forms, activation, degranulation, and aggregation.[27,28] Metabolic changes include increased glycolysis with a consequential increase in lactate and decrease in pH.[27,28] Development of the storage lesion is associated with decreased in vivo recovery, survival, and function. The mechanisms of these changes are multifactorial and not well understood, but are known to be influenced by all stages of platelet processing including collection methods, storage media, storage containers, pathogen reduction, and leukoreduction.[27,28]

RT platelets are commonly stored either in plasma or in a combination of plasma and a platelet additive solution

(PAS). PAS have been developed to 1) reduce the storage lesion by optimizing metabolism and reducing activation; 2) avoid transfusion associated circulatory overload and transfusion related acute lung injury due to a reduction in plasma, and hence a reduction in volume and inflammatory mediators, respectively; and 3) allow plasma to be used to produce other blood products, such as fresh frozen plasma. A variety of PAS have been developed, so to avoid confusion a standard terminology has been adopted. Current solutions are labeled from PAS-A to PAS-G.[29,30] These all contain varying combinations of citrate, phosphate, acetate, magnesium, potassium, gluconate, and glucose, and synergistically act to provide anticoagulation, membrane stabilization, metabolic substrates, buffering activity, and activation inhibition.[29,30]

A number of novel additives to platelet storage media are under investigation. These aim to attenuate the development of the storage lesion by reversibly inhibiting activation or preventing the changes in pH and lactate associated with glycolysis.

Various methods for reversibly inhibiting activation during storage have been investigated. Omega-3 fatty acids inhibit platelet activation and aggregation, and these properties are restored when the platelets are washed.[31,32] After storage for 10 days, omega-3 treated platelets have better thrombus formation, higher thrombin generation, and better aggregation response in vitro.[33] Prostaglandin E1 (PGE1) inhibits platelet aggregation during storage that is reversed when the platelets are resuspended in a PGE1 free media.[34] However, earlier studies found reduced in vivo survival of PGE1 treated platelets after 5 days of storage, and note that the inhibitory effects of PGE1 are lost after 5 days of storage.[35] Other reversible inhibitors of platelet activation have been investigated, including GPIIb-IIIa blockade,[36] a polyphenol derived from green tea,[37] nitric oxide,[38] and the phosphodiesterase inhibitor theophylline.[39]

Normally, platelets derive most of their energy from ß-oxidation of fatty acids.[40] During storage, ß-oxidation shifts to glycolysis, possibly due to mitochondrial dysfunction, with resulting increased lactate production and an associated decrease in pH, as well as reduced production of ATP.[41,42] L-carnitine facilitates transport of long chain fatty acids across the mitochondrial membrane.[43] Theoretically, adding L-carnitine to platelet storage media could increase ß-oxidation and decrease the glycolysis related adverse effects. L-carnitine also inhibits the apoptosis mediator Caspase-3, suggesting it could also reduce platelet apoptosis.[44] In vitro studies have found that addition of L-carnitine to storage media does result in decreased lactate production and glucose consumption, with an associated preservation of pH after 2 to 5 days of storage.[45,46] This is associated with an improvement in other in vitro markers of platelet function including shape change, HSR, and platelet counts after 5 days of storage,[46] and an improved aggregation response sometime between 2 and 5 days of storage.[45,46] Therefore, supplementation of platelet storage media with L-carnitine may be beneficial for platelets stored at RT for longer than 2 days, with maximal benefit seen if RT storage is extended beyond 5 days.

Another approach to attenuate the platelet storage lesion is to target signaling pathways, such as the one that is responsible for shedding of the GPIba receptor. A component of the complex that is responsible for von Willebrand factor (vWF) binding, GPIba is shed by a metalloproteinase that is activated by the p38 mitogen activated protein kinase (MAPK) pathway.[47] Inhibition of p38MAPK decreases shedding of GPIba with an associated improvement in platelet survival in a mouse model, as well as a reduction in activation, improved metabolic markers, improved morphological parameters, and improved HSR in vitro.[47,48] An endogenous endocannabinoid that inhibits platelet apoptosis via the PI3K/Akt pathway demonstrated improved pH, P-selectin expression and HSR, but only by Day 7 of storage.[49] Targeting other signaling pathways such as the one responsible for GPIIb-IIIa activation has also been suggested.[50] However, the platelet storage lesion is multifactorial, and so the clinical relevance of targeting one receptor or pathway remains to be seen, but at the very least these experiments provide interesting mechanistic data.

Improvements in PAS for RT platelet storage may lead to a product that can be stored longer while retaining morphology and function. However, an important consideration is that any inhibitory effect during storage must be fully reversible on transfusion or hemostatic function is likely to be impaired. In fact, a certain degree of pre-activation is thought to convey superior hemostatic properties, as partially activated platelets are able to rapidly participate in clot formation.[51] While the initial in vitro data on these novel PAS is promising, in vivo and eventually clinical studies are required to determine if the benefits seen in vitro translate to improved clinical outcomes. This should include an evaluation of hemostatic function in addition to the circulation survival time.

Cold

Storage of platelets at 4°C (cold) was largely abandoned in the 1980s in favor of the longer circulation time of RT stored platelets.[7] Cold stored platelets are cleared rapidly from the circulation by hepatic macrophages, resulting in a half-life of around 1.3 days compared to the 3.9 days of RT stored platelets.[7,20] They also undergo a number of changes collectively described as the cold storage lesion. These consist of an irreversible disc-to-sphere shape change, apoptosis, and evidence of activation including increased thromboxane A2 production and increased surface expression of P-selectin and GPIba.[29,52,53] However, precisely because of this degree of pre-activation, cold stored platelets may be more effective than RT platelets at

achieving hemostasis. Many in vitro and in vivo studies have demonstrated that cold stored platelets have better adhesion and aggregation functionality than RT platelets, which is associated with a reduction in bleeding times.[51,54,55] Cold stored platelets also suffer less bacterial contamination, do not need to be agitated, are metabolically quiescent with minimal lactate accumulation, and contain fewer inflammatory mediators than RT platelets.[51,54,56]

Novel methods for improving the viability of platelets after cold storage are under investigation, including metabolic suppression with a glucose free medium and inhibition of mitochondrial ATP synthesis,[57] and are reviewed elsewhere in this supplement.

Cold stored platelets would be easy to integrate into blood product supply chains as red blood cells are currently stored under similar conditions. However, they are unlikely to be suitable for prophylactic transfusion for thrombocytopenia due to their relatively short circulation time. Maintaining dual inventories of both cold and RT platelets may be challenging, but ultimately necessary if clinical trials demonstrate that cold stored platelets are a superior resuscitation product for active hemorrhage.

Temperature cycled

McGill first explored the effects of temperature cycling (TC) on platelet storage in the 1970s.[58] Despite promising early results that suggested an improvement in viability and function over cold (4°C) and RT (22°C) storage,[58] the idea appears to have fallen by the wayside as RT storage became the standard practice. In recent years, there has been a minor resurgence of interest in TC primarily as a way to reduce bacterial growth in stored platelets, but there is also further evidence that TC stored platelets may improve post transfusion viability.

The cold storage lesion is reversible if cold exposure is less than around 12 to 18 continuous hours.[59,60] In TC, continuous cold storage is periodically interrupted with brief rewarming phases that prevent the irreversible development of the cold storage lesion. McGill demonstrated that by rewarming cold (4°C) stored platelets to 37°C for 30 minutes every 12 hours microtubule reassembly function was preserved, there was a normal aggregation response and recovery from osmotic shock was better than continuous cold and RT stored platelets.[61] Further studies have shown that the microtubule and actin filament changes that are responsible for the loss of discoid shape during cold storage are reversible on rewarming after a short duration of cold storage.[62] McGill went on to study radiolabelled platelets in vivo and reported that platelets stored for 72 hours at 4°C had a 2-day life span compared to a 5.4-day life span for platelets TC for 72 hours,[58] however, this was only reported in abstract form and details of the experiment are not given. In 2013, Xu et al. reported similar findings in vivo after transfusing TC (cycled to 37°C for 30 min every 12 hr of cold storage), RT and cold stored human platelets into immunodeficient mice. For platelets that were stored for 2 days, RT storage produced the highest in vivo recovery rates, followed by TC and then cold storage. But when platelets were stored for either 5 or 7 days, those stored with TC had better in vivo recovery rates than both RT and cold stored platelets.[60] In contrast, Skripchenko et al. reported comparable in vivo recoveries of TC platelets as RT stored platelets after 7 days of storage, but superior to cold stored platelets.[63]

TC reduces bacterial proliferation in stored platelets. In the Xu et al. study, *Staphylococcus aureus* and *Klebsiella pneumonia* did not proliferate in cold or TC platelets stored for 48 hours, while there was exponential growth in RT stored platelets.[60] Wagner et al. went on to test 11 bacterial species and found that while cold storage prevented the growth of all but one (*Pseudomonas fluorescens*) of the tested bacterial species, only seven species were prevented from growing by TC the platelets, although the growth of the remaining four species was substantially inhibited compared to the RT stored platelets.[64]

TC may prove to provide the best of both worlds. That is, the superior recovery and survival rates of RT platelets, but with the preserved function, reduced metabolic activity and inhibition of bacterial growth of cold stored platelets. However, while promising, the data so far is very limited, so further work is needed to corroborate and expand on the current evidence, and in particular to further explore the hemostatic function of TC platelets as it pertains to the treatment of active hemorrhage.

CRYOPRESERVED PLATELETS

Cryopreservation of platelets using dimethylsulfoxide (DMSO) was developed by the US Navy in the 1970s.[65] The initial approach involved freezing platelets suspended in DMSO solution with post-thaw removal of the DMSO. Removing most of the DMSO before freezing, allowing omission of any post-thaw processing, was found to produce a comparable product[66] with obvious advantages in rapidly delivering the product to the patient with minimal equipment and training required in the hospital blood bank. A modification of this technique (resuspending platelets in plasma rather than saline) was operationalized by the Dutch military in Bosnia and Afghanistan.[67]

In vitro and ex vivo studies of cryopreserved platelets show promising results. In a baboon model, 54% of thawed cryopreserved platelets were recovered 2 hours after transfusion, more than was true for liquid platelets stored for 5 days.[68] Cryopreserved human platelets have a higher capacity to bind factor V[69] than liquid stored platelets, and produce more thromboxane A2 after ADP stimulation.[70] In Phase I, human studies involving a total of 32

healthy volunteers,[71] cryopreserved platelets obtained by apheresis and stored at <65°C were compared with fresh liquid-stored apheresis platelets. Radiolabelling allowed assessment of posttransfusion viability. When thawed, in the subset of 24 patients in whom this was reported,[72] 82% of cryopreserved platelets were viable. Twenty-four hours after transfusion, fewer cryopreserved platelets remained in the circulation (mean 33 vs. 63%, $p < 0.001$), but mean duration of circulation was only slightly (but still statistically significantly) reduced (7.5 vs. 8.6 days, $p < 0.001$), comparable to the results of the study of seven healthy volunteers in which the technique was initially described.[66]

There are few human clinical studies of bleeding patients transfused cryopreserved platelets. In total, there are literature reports of 868 trauma patients having received 1679 cryopreserved platelet units, apparently effectively and without adverse events[71]—although without comparator groups these reports are at best encouraging. In a prospective audit of 46 patients who received massive transfusions, and 234 patients who received less-than-massive transfusions in a NATO military hospital in Afghanistan, receipt of a high ratio of cryopreserved platelets to red blood cells (>= 1:8) compared to a lower ratio was associated with increased survival in the massive transfusion group (74 vs. 50%), and no difference in survival in the less-than-massive transfusion group (86 vs. 90%).[73] The single controlled clinical trial of thawed frozen platelets randomized 73 patients to cryopreserved or liquid-stored platelets if required for treatment of bleeding after cardiac surgery.[74] Blood loss in the 24 patients who received cryopreserved platelets was significantly less than in the 29 patients who received liquid-stored platelets, despite lower posttransfusion platelet increments and a tendency toward decreased platelet survival. There was no observable difference in adverse effects between the groups.

A postulated mechanism for greater hemostatic efficacy was that the DMSO/freeze/thaw process had "preactivated" the platelets so that they bound more rapidly to the damaged endothelium following transfusion. This hypothesis was supported by in vitro assays that found the cryopreserved platelets produced more thromboxane A2 and generated more procoagulant activity on their surface (as determined by flow cytometry analysis of the binding of an activated coagulation factor V—specific monoclonal antibody) in response to stimulation, congruent with earlier work that found cryopreservation increased the ability of platelets to activate coagulation.[69,70] More recently, the formation of phosphatidylserine and tissue-factor expressing platelet microparticles has been recognized as a significant contributor to the hemostatic effect of cryopreserved platelets.[75-77] Thawed cryopreserved platelet units contain a 15-fold higher concentration of functional platelet microparticles compared to fresh and Day 5 RT stored platelets.[75,76] The microparticle containing supernatant of cryopreserved platelets reduces clotting time and stimulates a twofold increase in phosphatidylserine and tissue factor induced peak thrombin generation compared to fresh platelet supernatant.[75,76] Filtering the microparticles from the supernatant substantially reduces this effect.[75] Furthermore, the clotting time of the cryopreserved supernatant is similar to cryopreserved platelet concentrates, which suggests microparticles are the key mediator of the procoagulant activity of cryopreserved platelets.[77]

While the single center trial in which 24 patients received cryopreserved platelets found encouraging results, a number of questions and concerns remain. The sample size may have been underpowered to be sure of safety, particularly in the medium to long term. Indeed, outcomes beyond the immediate perioperative period were not assessed. If the thrombus formed by cryopreserved platelets is more fragile, increasing the incidence of later bleeding, or if the apparent hypercoagulable state induced by cryopreserved platelets led to more graft occlusion or thromboembolic disease, this would not have been observed. DMSO is known to cause neurological, cardiac and renal toxicity, although this requires doses of >1.0 g/kg, 100 times higher than is present in a cryopreserved platelet unit (approx. 0.8 g/unit).[67] Many argue there is sufficient equipoise for a clinical trial in bleeding patients, such as that currently underway in Australian civilian cardiac surgical patients and sponsored by the Australian Red Cross Blood Service and the Australian Defence Force (Australian New Zealand Clinical Trials Registry ACTRN12612001261808).

LYOPHILIZED PLATELETS

A lyophilized, or freeze-dried, platelet product could potentially be the best approach to platelet storage, particularly for rural and remote environments. The ideal product would be light, temperature stable, have a long shelf life, and be rehydrated at the point of care with little processing. It would also need to be hemostatically functional but not cause thromboembolic complications, excess antigenicity or reticuloendothelial accumulation. Lyophilization of platelets was first attempted in the 1950s, but the rehydrated platelets were not structurally intact or hemostatically effective.[78,79] In the 1990s, the lyophilization process was improved by stabilizing the platelets with paraformaldehyde before freeze drying.[80] In vitro and in vivo preclinical investigations of the paraformaldehyde lyophilized platelets (pLP) have been encouraging.

After rehydration, pLP have near normal morphology.[80,81] Their adhesive properties also appear largely intact. They will adhere to exposed sub-endothelium even under conditions of high shear stress via physiological GPIb and vWF interactions, but to a lesser degree than

fresh whole blood or fresh platelets (FP).[80,82,83] Activation and aggregation functions, however, are impaired. They produce considerably less thromboxane and express less P-selectin after stimulation than FP, but there is still evidence of spreading, pseudopodia and degranulation when forming fibrin clots.[80,84] GPIIb-IIIa is constitutively expressed on rehydrated pLP at half the density of stimulated FP, owing to partial activation during the lyophilization process, and no further increase in GPIIb-IIIa expression is seen after stimulation.[83-86] Fibrinogen binding to pLP is functional, and is mediated physiologically via binding to GPIIb-IIIa, but with around half the affinity of FP.[83,84,87] Rehydrated pLP retain the ability to facilitate coagulation. Stimulated and unstimulated pLP support thrombin production at a similar rate to each other and to stimulated pLP.[83,84,88] Factor V and rFVIIa bind readily to pLP.[85,86,89]

Animal studies have gone on to demonstrate hemostatic efficacy of pLP in vivo. Infusion of pLP into bleeding thrombocytopenic rats, swine, rabbits, and dogs improved bleeding time.[80,90-92] Fluorescently labeled canine pLP localized to the site of vascular injury in dogs and participated in thrombus formation.[80] In a randomized controlled trial of 37 actively bleeding dogs, there was no difference between animals that received canine pLP or FP in transfusion requirements, ongoing bleeding at 24 hours, platelet increment, adverse reactions or survival.[93] In a swine model of uncontrolled liver hemorrhage, an infusion of human pLP resulted in rapid hemostasis but with a high rate of severe prothrombotic complications.[94] However, porcine vWF has a high affinity for human platelet GPIb and will induce activation and aggregation of human platelets in the absence of agonists, so it is possible that the high prothrombotic complications are a result of poor xenotransplantation compatibility.[95] In contrast, in a different swine model of nonsurgical bleeding there was poor hemostasis with the human pLP, possibly due to aggregation within the circulation before vascular injury localization, and no prothrombotic complications, while porcine pLP achieved superior hemostasis.[96] These studies suggest that swine are a poor model for studying human platelets.

Other evidence does not support a high risk of prothrombotic complications after transfusion of pLP. In vivo studies report that pLP have minimal adhesion to intact endothelium.[80,82] They are rapidly cleared from the circulation after transfusion predominantly via IgG mediated macrophage phagocytosis.[86,97] Therefore, pLP that are not bound immediately to sites of vascular injury are removed, reducing the potential for later prothrombotic complications, although they are likely to accumulate in the spleen.[97,98] In addition, agents that antagonize the GPIIb-IIIa receptor are clinically effective at reducing thrombosis during percutaneous coronary interventions.[99] It is therefore unlikely that transfusion of pLP, with their poor GPIIb-IIIa activity, would lead to an increased risk of prothrombotic complications.

More recently, another methodology has been described where platelets are loaded with trehalose before lyophilization.[100] When rehydrated, these platelets have a recovery of around 80%, an increased mean platelet volume compared to FP, and around 40% of cells expressed P-selectin and GPIIb-IIIb indicating pre-activation.[101] Intracellular pH regulation is functional.[102] They have a very similar clot formation time to FP,[100] and the maximum aggregation rate is around 80% of FP, with the aggregation speed around 40% of FP.[103]

These initial results suggest that lyophilized platelets may have a role in the treatment of acute hemorrhage, but not in the prophylactic management of thrombocytopenia due to their rapid clearance from the circulation. The current lyophilization methods produce a product with inferior hemostatic properties than FP, but they may still have a role where FP are not available provided an acceptable safety profile can be established in human clinical trials.

CONCLUSION

RT storage has been the status quo of platelet storage for over 30 years. When first introduced, it was in an era where platelets were transfused primarily to oncology and hematology patients prophylactically, and hemostatic function was sacrificed for circulating time. Now that the importance of a resuscitation strategy for active hemorrhage that includes platelet transfusion has become well established, it is timely to review platelet storage methodology. The best storage method for achieving hemostasis in active hemorrhage may not be the most appropriate method for other indications, and the possibility of split platelet inventories may need to be explored. Logistic considerations may mandate exploration of a different product again for rural and remote facilities where efficacy may need to be sacrificed for storage life. Maintaining the status quo of platelet storage may commit those hemorrhaging patients who receive RT stored platelets to a suboptimal resuscitation product, and deny those in rural and remote facilities access to a potentially life-saving therapy.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION.

REFERENCES

1. Kautza BC, Cohen MJ, Cuschieri J, et al. Changes in massive transfusion over time: an early shift in the right direction? J Trauma Acute Care Surg 2012;72:106-11.
2. Pidcoke HF, Aden JK, Mora AG, et al. Ten-year analysis of transfusion in operation Iraqi freedom and operation enduring freedom: increased plasma and platelet use

correlates with improved survival. J Trauma Acute Care Surg 2012;73:S445-52.
3. Hallet J, Lauzier F, Mailloux O, et al. The use of higher platelet: RBC transfusion ratio in the acute phase of trauma resuscitation: a systematic review. Crit Care Med 2013;41:2800-11.
4. Holcomb JB, Wade CE, Michalek JE, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. Ann Surg 2008;248:447-58.
5. Holcomb JB, del Junco DJ, Fox EE, et al. The prospective, observational, multicenter, major trauma transfusion (PROMMTT) study: comparative effectiveness of a time-varying treatment with competing risks. JAMA Surg 2013; 148:127-36.
6. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471-82.
7. Murphy S, Gardner FH. Platelet preservation—effect of storage temperature on maintenance of platelet viability - deleterious effect of refrigerated storage. N Engl J Med 1969;280:1094-8.
8. Valeri CR. Hemostatic effectiveness of liquid-preserved and previously frozen human platelets. N Engl J Med 1974;290: 353-8.
9. Murphy S, Gardner FH. Room temperature storage of platelets. Transfusion 1976;16:2-3.
10. Brecher ME, Blajchman MA, Yomtovian R, et al. Addressing the risk of bacterial contamination of platelets within the united states: a history to help illuminate the future. Transfusion 2013;53:221-31.
11. Dumont LJ, Gulliksson H, van der Meer PF, et al. Interruption of agitation of platelet concentrates: a multicenter in vitro study by the BEST collaborative on the effects of shipping platelets. Transfusion 2007;47:1666-73.
12. Cardigan R, Turner C, Harrison P. Current methods of assessing platelet function: relevance to transfusion medicine. Vox Sang 2005;88:153-63.
13. Holme S. Storage and quality assessment of platelets. Vox Sang 1998;74 Suppl 2:207-16.
14. Picker SM. In-vitro assessment of platelet function. Transfus Apher Sci 2011;44:305-19.
15. Lindahl TL, Ramstrom S. Methods for evaluation of platelet function. Transfus Apher Sci 2009;41:121-5.
16. Panzer S, Jilma P. Methods for testing platelet function for transfusion medicine. Vox Sang 2011;101:1-9.
17. Shams Hakimi C, Hesse C, Wallen H, et al. In vitro assessment of platelet concentrates with multiple electrode aggregometry. Platelets 2015;26:132-7.
18. Valeri CR, Feingold H, Marchion LD. Relation between response to hypotonic stress and CR-51 recovery in vivo of preserved platelets. Transfusion 1974;14:331-7.
19. Kim BK, Baldini MG. Platelet response to hypotonic shock—its value as an indicator of platelet viability after storage. Transfusion 1974;14:130-8.
20. Hoffmeister KM, Felbinger TW, Falet H, et al. The clearance mechanism of chilled blood platelets. Cell 2003;112: 87-97.
21. Gorlinger K, Shore-Lesserson L, Dirkmann D, et al. Management of hemorrhage in cardiothoracic surgery. J Cardiothorac Vasc Anesth 2013;27:S20-34.
22. Romlin BS, Soderlund F, Wahlander H, et al. Platelet count and function in paediatric cardiac surgery: a prospective observational study. Br J Anaesth 2014;113:847-54.
23. Klinger MHF. The storage lesion of platelets: ultrastructural and functional aspects. Ann Hematol 1996;73:103-12.
24. Paglia G, Sigurjonsson OE, Rolfsson O, et al. Comprehensive metabolomic study of platelets reveals the expression of discrete metabolic phenotypes during storage. Transfusion 2014;54:2911-23.
25. Paglia G, Palsson BO, Sigurjonsson OE. Systems biology of stored blood cells: can it help to extend the expiration date? J Proteomics 2012;76 Spec No.:163-7.
26. Prudent M, Tissot JD, Lion N. Proteomics of blood and derived products: what's next? Expert Rev Proteomics 2011; 8:717-37.
27. Devine DV, Serrano K. The platelet storage lesion. Clin Lab Med 2010;30:475-87.
28. Seghatchian J, Krailadsiri P. The platelet storage lesion. Transfus Med Rev 1997;11:130-44.
29. Capocelli KE, Dumont LJ. Novel platelet storage conditions: additive solutions, gas, and cold. Curr Opin Hematol 2014; 21:491-6.
30. Gulliksson H. Platelet storage media. Vox Sang 2014;107: 205-12.
31. Spinelli SL, Casey AE, Lannan KL, et al. Improving the efficacy and safety of platelet transfusion: novel naturally occurring and physiological agents as additives to mitigate the platelet storage lesion. Transfusion 2013;53:84A-A.
32. Krishnamurti C, Stewart MW, Cutting MA, et al. Assessment of omega-fatty-acid-supplemented human platelets for potential improvement in long-term storage. Thromb Res 2002;105:139-45.
33. Stivala S, Meyer SC, Reiner M, et al. Addition of Omega-3 alpha-linolenic acid to platelet apheresis units preserves platelet activatability over time and reduces baseline activation under routine storage conditions: a pilot study. Blood 2012;120:3433.
34. Segawa K, Kondo T, Kimura S, et al. Effects of prostaglandin E1 on the preparation of platelet concentrates in dogs. J Vet Intern Med 2012;26:370-6.
35. Hawker RJ, Turner VS, Mitchell SG. Use of prostaglandin E(1) during preparation of platelet concentrates. Transfus Med 1996;6:249-54.
36. Jennings LK, White M, Cholera S, et al. Reversible blockade of GPIIb-IIIa reduces platelet storage lesions. J Thromb Haemostasis 2013;11:510.
37. Matsumura K, Takayama H, Bae JY, et al. Preservation of platelets by adding Epigallocatechin-3-O-gallate to platelet concentrates. Cell Transplant 2009;18:521-8.

38. Wong K, Li XB. Nitric oxide infusion alleviates cellular activation during preparation, leukofiltration and storage of platelets. Transfus Apher Sci 2004;30:29-39.
39. Bode AP, Norris HT. The use of inhibitors of platelet activation or protease activity in platelet concentrates stored for transfusion. Blood Cells 1992;18:361-80, discussion 381-2.
40. Donabedi. R, Nemerson Y. Fatty acid oxidation by human platelets and its stimulation by thrombin. Am J Physiol 1971;221:1283.
41. Sweeney JD, Arduini A. L-carnitine and its possible role in red cell and platelet storage. Transfus Med Rev 2004;18:58-65.
42. Murphy S. The oxidation of exogenously added organic-anions by platelets facilitates maintenance of pH during their storage for transfusion at 22 degrees C. Blood 1995;85: 1929-35.
43. Pekala J, Patkowska-Sokola B, Bodkowski R, et al. L-carnitine-metabolic functions and meaning in humans life. Curr Drug Metab 2011;12:667-78.
44. Mutomba MC, Yuan H, Konyavko M, et al. Regulation of the activity of caspases by L-carnitine and palmitoylcarnitine. FEBS Lett 2000;478:19-25.
45. Deyhim MR, Mesbah-Namin SA, Yari F, et al. L-carnitine effectively improves the metabolism and quality of platelet concentrates during storage. Ann Hematol 2015;94:671-80.
46. Sweeney JD, Blair AJ, Cheves TA, et al. L-carnitine decreases glycolysis in liquid-stored platelets. Transfusion 2000;40: 1313-9.
47. Canault M, Duerschmied D, Brill A, et al. p38 mitogen-activated protein kinase activation during platelet storage: consequences for platelet recovery and hemostatic function in vivo. Blood 2010;115:1835-42.
48. Skripchenko A, Awatefe H, Thompson-Montgomery D, et al. An inhibition of p38 mitogen activated protein kinase delays the platelet storage lesion. PLoS One 2013;8:e70732.
49. Zhuang YL, Ren GJ, Li HY, et al. In vitro properties of apheresis platelet during extended storage in plasma treated with anandamide. Transfus Apher Sci 2014;51:58-64.
50. Schubert P, Thon JN, Walsh GM, et al. A signaling pathway contributing to platelet storage lesion development: targeting PI3-kinase-dependent Rap1 activation slows storage-induced platelet deterioration. Transfusion 2009;49:1944-55.
51. Pidcoke HF, Spinella PC, Ramasubramanian AK, et al. Refrigerated platelets for the treatment of acute bleeding: a review of the literature and reexamination of current standards. Shock 2014;41 Suppl 1:51-3.
52. van der Wal DE, Du VX, Lo KS, et al. Platelet apoptosis by cold-induced glycoprotein ibalpha clustering. J Thromb Haemost 2010;8:2554-62.
53. van der Wal DE, Gitz E, Du VX, et al. Arachidonic acid depletion extends survival of cold-stored platelets by interfering with the [glycoprotein ibalpha-14-3-3zeta] association. Haematologica 2012;97:1514-22.
54. Reddoch KM, Pidcoke HF, Montgomery RK, et al. Hemostatic function of apheresis platelets stored at 4 degrees C and 22 degrees C. Shock 2014;41:54-61.
55. Montgomery RK, Reddoch KM, Evani SJ, et al. Enhanced shear-induced platelet aggregation due to low-temperature storage. Transfusion 2013;53:1520-30.
56. Kaufman RM. Uncommon cold: could 4 degrees C storage improve platelet function? Transfusion 2005;45:1407-12.
57. Badlou BA, Ijseldijk MJ, Smid WM, et al. Prolonged platelet preservation by transient metabolic suppression. Transfusion 2005;45:214-22.
58. McGill M, Kahn RA, Genco PV. Temperature cycling improves cold liquid storage of platelets. Transfusion 1978; 18:640.
59. McGill M. Temperature cycling preserves platelet shape and enhances in vitro test-scores during storage at 4-degrees. J Lab Clin Med 1978;92:971-82.
60. Xu F, Gelderman MP, Farrell J, et al. Temperature cycling improves in vivo recovery of cold-stored human platelets in a mouse model of transfusion. Transfusion 2013;53:1178-86.
61. McGill M. Platelet storage by temperature cycling. Prog Clin Biol Res 1978;28:119-39.
62. White JG, Rao GHR. Microtubule coils versus the surface membrane cytoskeleton in maintenance and restoration of platelet discoid shape. Am J Pathol 1998;152:597-609.
63. Skripchenko A, Vostal JG, Gelderman MP, et al. Human platelets, stored in an automated temperature cycler between 4.5 C and 37 C for 7 days, exhibited improved in vitro properties and in vivo recovery in SCID mice compared to continuous cold storage. Transfusion 2013;53:72A.
64. Wagner SJ, Seetharaman S, Cook T. Evaluation of bacterial growth during storage of apheresis platelets with temperature cycling. Transfusion 2014;54:204A.
65. Valeri CR, Feingold H, Marchionni LD. A simple method for freezing human platelets using 6 per cent dimethylsulfoxide and storage at −80 degrees C. Blood 1974;43:131-6.
66. Valeri CR, Ragno G, Khuri S. Freezing human platelets with 6 percent dimethyl sulfoxide with removal of the supernatant solution before freezing and storage at −80 degrees C without postthaw processing. Transfusion 2005;45:1890-8.
67. Lelkens CC, Koning JG, de Kort B, et al. Experiences with frozen blood products in the netherlands military. Transfus Apher Sci 2006;34:289-98.
68. Valeri CR, Giorgio A, Macgregor H, et al. Circulation and distribution of autotransfused fresh, liquid-preserved and cryopreserved baboon platelets. Vox Sang 2002;83:347-51.
69. Barnard MR, MacGregor H, Ragno G, et al. Fresh, liquid-preserved, and cryopreserved platelets: adhesive surface receptors and membrane procoagulant activity. Transfusion 1999;39:880-8.
70. Valeri CR, Macgregor H, Ragno G. Correlation between in vitro aggregation and thromboxane A2 production in fresh, liquid-preserved, and cryopreserved human platelets: effect of agonists, pH, and plasma and saline resuspension. Transfusion 2005;45:596-603.
71. Slichter SJ, Jones M, Ransom J, et al. Review of in vivo studies of dimethyl sulfoxide cryopreserved platelets. Transfus Med Rev 2014;28:212-25.

72. Dumont LJ, Cancelas JA, Dumont DF, et al. A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide-frozen autologous platelets in healthy volunteers. Transfusion 2013;53:128-37.
73. Noorman FB. Efficient logistics available, compatible, safe and effective in the treatment of trauma patients with or without massive blood loss in military theatre. Transfusion 2012;52:S53-030H.
74. Khuri SF, Healey N, MacGregor H, et al. Comparison of the effects of transfusions of cryopreserved and liquid-preserved platelets on hemostasis and blood loss after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1999;117: 172-83, discussion 183-4.
75. Raynel S, Padula MP, Marks DC, et al. Cryopreservation alters the membrane and cytoskeletal protein profile of platelet microparticles. Transfusion 2015;55:2422-32.
76. Johnson L, Reade MC, Hyland RA, et al. In vitro comparison of cryopreserved and liquid platelets: potential clinical implications. Transfusion 2015;55:838-47.
77. Johnson L, Coorey CP, Marks DC. The hemostatic activity of cryopreserved platelets is mediated by phosphatidylserine-expressing platelets and platelet microparticles. Transfusion 2014;54:1917-26.
78. Fliedner TM, Sorensen DK, Bond VP, et al. Comparative effectiveness of fresh and lyophylized platelets in controlling irradiation hemorrhage in the rat. Proc Soc Exp Biol Med 1958;99:731-3.
79. Jackson DP, Sorensen DK, Cronkite EP, et al. Effectiveness of transfusions of fresh and lyophylized platelets in controlling bleeding due to thrombocytopenia. J Clin Invest 1959; 38:1689-97.
80. Read MS, Reddick RL, Bode AP, et al. Preservation of hemostatic and structural properties of rehydrated lyophilized platelets—potential for long-term storage of dried platelets for transfusion. Proc Natl Acad Sci U S A 1995;92:397-401.
81. Joshi NV, Raftis JB, Lucking AJ, et al. Lyophillised reconstituted unman platelets increase thrombus formation in a clinical ex vivo model of deep arterial injury. Thromb Haemost 2012;108:176-82.
82. Bode AP, Read MS, Reddick RL. Activation and adherence of lyophilized human platelets on canine vessel strips in the baumgartner perfusion chamber. J Lab Clin Med 1999; 133:200-11.
83. Fischer TH, Bode AP, Parker BR, et al. Primary and secondary hemostatic functionalities of rehydrated, lyophilized platelets. Transfusion 2006;46:1943-50.
84. Fischer TH, Merricks EP, Bode AP, et al. Thrombus formation with rehydrated, lyophilized platelets. Hematology 2002;7:359-69.
85. Valeri CR, Macgregor H, Barnard MR, et al. In vitro testing of fresh and lyophilized reconstituted human and baboon platelets. Transfusion 2004;44:1505-12.
86. Valeri CR, MacGregor H, Barnard MR, et al. Survival of baboon biotin-X-N-hydroxysuccinimide and in-111-oxine-labelled autologous fresh and lyophilized reconstituted platelets. Vox Sang 2005;88:122-9.
87. Sanders WE, Khandelwal G, Bellinger DA, et al. Fibrinogen binding to rehydrated lyophilize platelet surface GpIIb-IIIa: normal clot formation and tPA lysts. Blood 1996;88:107.
88. Campbell RA, Fischer TH, Wolberg AS. A novel approach to improving recombinant factor VIIa activity with a preserved platelet preparation. Br J Haematol 2007;138:82-93.
89. Fischer TH, Wolberg AS, Bode AP, et al. The interaction of factor VIIa with rehydrated, lyophilized platelets. Platelets 2008;19:182-91.
90. Bode AP, Lust RM, Read MS, et al. Correction of the bleeding time with lyophilized platelet infusions in dogs on cardiopulmonary bypass. Clin Appl Thromb Hemost 2008;14:38-54.
91. Fischer TH, Merricks EP, Nichols TC, et al. The co-infusion of rehydrated, lyophilized platelets with HBOC-201 for hemostasis in dilutional thrombocytopenia. Blood 2001;98:538A.
92. Bode AP, Blajchman M, Bardossy L, et al. Hemostatic properties of human lyophilized platelets in a thrombocytopenic rabbit model and a simulated bleeding-time device. Blood 1994;84:A464-A.
93. Davidow EB, Brainard B, Martin LG, et al. Use of fresh platelet concentrate or lyophilized platelets in thrombocytopenic dogs with clinical signs of hemorrhage: a preliminary trial in 37 dogs. J Vet Emerg Crit Care 2012;22:116-25.
94. Hawksworth JS, Elster EA, Fryer D, et al. Evaluation of lyophilized platelets as an infusible hemostatic agent in experimental non-compressible hemorrhage in swine. J Thromb Haemost 2009;7:1663-71.
95. Pareti FI, Mazzucato M, Bottini E, et al. Interaction of porcine von Willebrand factor with the platelet glycoproteins ib and IIb/IIIa complex. Br J Haematol 1992;82:81-6.
96. Inaba K, Barmparas G, Rhee P, et al. Dried platelets in a swine model of liver injury. Shock 2014;41:429-34.
97. Fischer TH, Merricks E, Bellinger DA, et al. Splenic clearance mechanisms of rehydrated, lyophilized platelets. Artif Cells Blood Substit Immobil Biotechnol 2001;29:439-51.
98. Cap AP, Perkins JG. Lyophilized platelets: challenges and opportunities. J Trauma 2011;70:S59-60.
99. Muniz-Lozano A, Rollini F, Franchi F, et al. Update on platelet glycoprotein IIb/IIIa inhibitors: recommendations for clinical practice. Ther Adv Cardiovasc Dis 2013;7:197-213.
100. Wolkers WF, Walker NJ, Tablin F, et al. Human platelets loaded with trehalose survive freeze-drying. Cryobiology 2001;42:79-87.
101. Wang JX, Yang C, Wang Y, et al. Stability of lyophilized human platelets loaded with small molecule carbohydrates. Cryoletters 2011;32:123-30.
102. Tang MK, Wolkers WF, Crowe JH, et al. Freeze-dried rehydrated human blood platelets regulate intracellular pH. Transfusion 2006;46:1029-37.
103. Zhou XL, Zhu H, Zhang SZ, et al. Freeze-drying of human platelets: influence of saccharide, freezing rate and cell concentration. Cryoletters 2007;28:187-96.