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Towards increasing shelf life and haemostatic potency of stored platelet concentrates

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Abstract

Purpose of review—Platelet transfusion is a widely used therapy in treating or preventing bleeding and haemorrhage in patients with thrombocytopenia or trauma. Compared with the relative ease of platelet transfusion, current practice for the storage of platelets is inefficient, costly and relatively unsafe, with platelets stored at room temperature (RT) for upto 5–7 days.

Recent findings—During storage, especially at cold temperatures, platelets undergo progressive and deleterious changes, collectively termed the 'platelet storage lesion', which decrease their haemostatic function and posttransfusion survival. Recent progress in understanding platelet activation and host clearance mechanisms is leading to the consideration of both old and novel storage conditions that use refrigeration and/or cryopreservation to overcome various storage lesions and significantly extend platelet shelf-life with a reduced risk of pathogen contamination.

Summary—A review of the advantages and disadvantages of alternative methods for platelet storage is presented from both a clinical and biological perspective. It is anticipated that future platelet preservation involving cold, frozen and/or pathogen reduction strategies in a proper platelet additive solution will enable longer term and safer platelet storage.

Keywords

'cold storage lesion'; bacterial contamination; platelet shelf life

INTRODUCTION

Five million platelet concentrates doses are estimated to be administered every year in the USA and Europe. In the USA, where approximately 40% of all platelet doses are

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Conflicts of interest

Y.Z. and J.A.C. own intellectual property of US Patent US20150366182A1 and international related protections. J.A.C. has received research funding from the US DoD, Cellphire, Cerus Co. and TerumoBCT for projects related to developing novel platelet products. No other relevant conflicts of interest are reported.

administered, the average dose for an adult transfusion consists of 300–400 billion platelets, which is equivalent to the amount in four to five whole blood derived collections or one apheresis collection [1].

Platelets are collected in three different ways. Platelets can be centrifuged from platelet-rich plasma, isolated from buffy coats or collected directly from the bloodstream by apheresis. There is some evidence that the buffy coat and apheresis methods provide better platelets, because it is thought that centrifuging platelets in the platelet-rich plasma method lead to partial or complete activation of some of the platelets [2]. The current most prevalent method for platelet storage is room temperature with continuous agitation (RT-PLT).

Current strategies have significantly reduced the probability of bacterial contamination in platelet products. The use of diversion pouches during collection or moderately sensitive bacterial detection with automatic culture systems has been implemented [3,4,5,6,7]. Despite these advances, the risk of platelet transfusion-associated bacteraemia in the USA ranges between 1 in 15 000–86 000 platelet transfusions with reported fatalities of one per 100 000–1 000 000 units transfused [8,9]. Implementation of pathogen reduction systems [10–12] has demonstrated that the safety profile of the platelet products can be modified [13,14,15–17]. Unfortunately, pathogen reduction treatment has not enabled us to extend the shelf-life of platelets beyond 7 days due to an activating effect on platelets that reduces their lifespan and therapeutic potency compared with untreated platelet products [18–21,22].

Despite significant advances in optimization of platelet concentrate storage methodologies, which include storage in large flat bags with a high surface to volume ratio and on agitators to facilitate oxygen diffusion and facilitate mitochondrial-dependent aerobic glycolysis [23,24], platelets undergo a collection of structural and functional changes that are referred to as the ‘platelet storage lesion’ (reviewed in [25]). These include reversible and irreversible changes that range from reversible disc-to-sphere shape change to irreversible breakdown to fragmented forms, activation, degranulation and aggregation [25]. Metabolic changes include increased glycolysis with a subsequent increase in lactate and decrease in pH. 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 influenced by all stages of platelet processing, including collection methods, storage media, storage containers, pathogen reduction and leukoreduction.

Current approaches to reduce the platelet lesion of RT-PLT platelets use a combination of plasma and a platelet additive solution (PAS), which provides significant advantages. First, PAS reduces the storage lesion by optimizing metabolism and reducing activation. Second, PAS reduces the frequency of 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. Finally, PAS allows blood banks to optimize fractionation by obtaining plasma to be used in other blood derivatives, such as fresh frozen plasma or plasma fractionation products.

A variety of PAS have been developed. Standardized nomenclature of PAS solutions labels them from PAS-A to PAS-G [26,27]. All these solutions 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 [23–26,28–32,33–34,35–39,40–41,42–43] (Table 1).

Several novel additives for platelet storage media are under investigation. These new solutions aim to attenuate the development of the storage lesion by reversibly inhibiting activation or preventing the changes in pH and lactate associated with glycolysis, or to reduce the risk of pathogen contamination.

FUNCTIONAL ANALYSIS OF PLATELET EFFICACY AND SAFETY

The roles of platelets in haemostasis and arterial thrombosis involve their adherence to sites of vessel injury or ruptured atherosclerotic plaques, aggregation to form haemostatic plugs or thrombi, and acceleration of the coagulation cascade leading to the formation of thrombin. Functional tests of platelet function are an integral part of platelet product development and therapies. Traditionally, in-vivo clinical bleeding assessments including past medical and family history, information on drug use affecting platelets and potential coagulation factor and tissue deficits are collected in the questionnaire process for platelet donation [44]. In-vitro functional assays for potency include: volume of platelet product, platelet content, mean platelet volume, levels of glucose, lactate, bicarbonate, pO_2 , pCO_2 , as well as activation measured by P-selectin, phosphatidylserine expression measured by annexin-V and lactadherin binding, morphology score, microparticle content, extracellular/intracellular ratio of lactic dehydrogenase, extent of shape change (ESC), hypotonic shock response (HSR), analysis of platelet aggregation by recording changes in light transmission, measurement of platelet-related haemostasis and platelet adherence and aggregation under conditions of high shear, measurement of secretion of granule contents, and formation of thromboxane B2 upon agonist stimulation [45,46]. The safety assessment of platelet products follows that recommended by the US Food and Drug Administration (FDA) and the American Society of Clinical Oncology (ASCO) for platelet transfusions in patients with cancer undergoing autologous stem-cell transplantation [47–49].

COLD PLATELET CONCENTRATES: ‘COLD STORAGE LESION’, ADVANTAGES AND DISADVANTAGES

Storage of platelets at 4°C was largely abandoned in the 1970s–1980s in favour of the longer circulation time of RT-PLT [48]. COLD-PLT are cleared rapidly from the circulation by hepatic macrophages, resulting in a half-life of approximately 1.3 days compared with the 4-day half-life of RT-PLT. COLD-PLT undergo additional changes, collectively described as the ‘cold storage lesion’. These consist of an irreversible disc-to-sphere shape change [49] and irreversible activation, including increased thromboxane A2 production and increased surface expression of P-selectin (reviewed in [28]).

However, a better understanding of the molecular mechanisms of platelet clearance has been elucidated in the last 15 years. Von Willebrand factor (vWF) binding, glycoprotein Iba (GPIba) clustering, platelet desialylation and phosphatidylserine exposure are key steps in the fast clearance of refrigerated platelets. COLD-PLT become desialylated following 48 h of refrigeration [29■■■]. This is because refrigeration and subsequent rewarming of the platelets induces surface expression of Neu1, a neuraminidase that removes sialic acid from multiple platelet glycoproteins, particularly GPIba. The exposed β -gal residues are recognized by the macrophage α M/ β 2 integrin receptor and the hepatocyte Ashwell–Morell receptor (AMR), and the platelets are quickly cleared in the liver and more modestly in the spleen [29■■■,30]. Incubation with the AMR inhibitor asia-lofetuoin significantly blocks the fast clearance of human refrigerated platelets [29■■■] and adding 2,3-dehydro-2-deoxy-*N*-acetylneuraminic acid (DANA), a neuraminidase inhibitor, to murine platelets during refrigeration improves the posttransfusion recovery and survival of refrigerated platelets after syngeneic transfusion [31]. The mechanism to explain this effect probably lies in the ability of refrigeration to induce binding of plasma VWF to GPIba on the platelet [29■■■, 32], as identified by the effect of O-sialoglycoprotein endopeptidase, which prevents the VWF-GPIba interaction and subsequent clustering of GPIba in refrigerated platelets [29■■■,33■■■].

A major hallmark of the ‘cold storage lesion’ is clustering of GPIba on the platelet surface [30]. In addition of being recognized by the receptors, GPIba clustering is thought to induce platelet apoptosis, as 14–3–3 ζ dissociates from the pro-apoptotic molecule Bad and associates with clustered GPIba following refrigeration, leading to the pro-apoptotic cascade of Bad activation, cytochrome C release, caspase 9 activation and phosphatidylserine exposure that results in increased macrophage-dependent phagocytosis [34]. This apoptotic process can be inhibited by caspase inhibition, or upstream by *N*-acetyl-D-glucosamine, which blocks platelet–macrophage interactions and potentially GPIba clustering. Furthermore, refrigeration-mediated 14–3–3 ζ -GPIba association depends on arachidonic acid, as the depletion of arachidonic acid during refrigeration inhibits apoptotic signals and results in improved posttransfusion recovery and survival of refrigerated platelets [35]. Finally, shear treatment of refrigerated wild-type platelets, but not vWF-deficient platelets, results in GPIba mechanosensory domain unfolding, platelet desialylation and phosphatidylserine exposure [33■■■], and refrigerated platelets incubated with a peptide that inhibits GPIba interaction with VWF, exhibit markedly higher posttransfusion recovery [33■■■].

Some studies have demonstrated that cold stored platelets have faster clot formation, although this was associated with increased microplatelet formation [50]. There is renewed interest in investigating the storage of platelets at refrigerated temperatures (1°C–6°C) to address these challenges [51–53] (Table 1). COLD-PLT aggregate better *in vitro* than RT-PLT, form stronger clots, preserve mitochondrial function better than RT-PLT, can be inhibited by physiologic antagonists, including nitric oxide and prostacyclin, and are functionally primed in static and flow assays. In addition, cold storage of platelets often inhibits cytokine secretion, thereby contributing to decreased cytokine-associated febrile transfusion reactions [54]. RT-PLT show increased platelet counts, preserved recovery/

survival properties and reduced aggregate formation during storage as compared with cold stored platelets in plasma [51–53].

In the USA, refrigerated platelet storage complies with the FDA criteria given in the Code of Federal Regulations (CFR) sections 21CFR610.53, 640.24 and 640.25 [55]. From the 1960s to the mid-1980s, COLD-PLT were a standard component for transfusion. Because of increased in-vivo survival, RT-PLT came to be in widespread use and COLD-PLT were abandoned [56,57]. However, RT-PLT have an increased risk of bacterial contamination [58,59] and may not be effective in rapid haemostatic function *in vivo* [60]. Recently, the FDA granted an exception to the CFR 606.65[e] and 610.53[c] to permit sites ‘to store apheresis platelets at refrigerator temperature (1°C–6°C) with or without agitation for up to 3 days’. These COLD-PLT will only be used in resuscitating actively bleeding patients because of data indicating COLD-PLT that these platelets aggregate better and have stronger clot formation than RT-PLT [51–53,61■■■]. Laboratory research has shown that platelets can be stored for longer than 3 days at refrigerator temperatures and maintain many of the properties needed for aggregation and clot formation [51–53]. A small number of clinical studies support the efficacy of refrigerated PLT-containing blood components [60,62,63■■■]. COLD-PLT better corrected the bleeding time than RT-PLT [60]. A study of aspirin-treated adults showed significantly better improvement of bleeding times after COLD-PLT as compared with RT-PLT. In fact, RT-PLT had a delayed and/or minimal effect on bleeding time [52]. A prospective, randomized, unblinded, noninferiority, two-arm study of leukocyte-reduced COLD-PLT in 60 patients undergoing cardiopulmonary bypass is ongoing in Norway (NCT02495506). In this trial, preliminary data indicate a nearly 30% reduction in chest drain output after chest closure during the procedure when using COLD-PLT, with no significant differences in the presence of thromboembolic events [64].

TEMPERATURE CYCLING STORAGE OF PLATELET CONCENTRATES

The ‘cold storage lesion’ is reversible if cold exposure is less than 12–18 continuous hours. Rewarming cold stored platelets to 37°C for 30min every 12 h preserved the microtubule reassembly, there was a normal aggregation response and the recovery from osmotic shock (HSR) was better than continuous cold- and RT-stored platelets, and extended the platelet survival *in vivo* [65,66]. In addition, 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 [67]. In recent years, groups at the FDA and the American Red Cross have reembraced this concept. They compared temperature cycled-PLT (TC-PLT) (cycling to 37°C for 30 min every 12 h of cold storage), RT-PLT and COLD-PLT, and compared their ability to prevent bacterial growth and survive after transfusion into immunodeficient mice. Both groups confirmed that TC-PLT have a reduced potential to become significantly contaminated during storage [68,69]. Regarding platelet survival, for platelets that were stored for 2 days, RT-PLT produced the highest in-vivo recovery rates, followed by TC-PLT and then COLD-PLT. But when platelets were stored for 5 or 7 days, TC-PLT had better in-vivo recovery rates than either RT-PLT or COLD-PLT [68]. The American Red Cross group confirmed part of these data, although they found comparable in-vivo recoveries for TC-PLT and RT-PLT after 7 days of storage, and were superior to COLD-PLT [69]. These results led to a clinical trial of autologous transfusion of

radiolabelled platelets into healthy volunteers, demonstrating that TC-PLT stored for 7 days in 100% plasma had better in-vivo circulation kinetics than COLD-PLT, but were not equivalent to RT-PLT [70■].

CRYOPRESERVED PLATELETS

Cryopreservation of platelets was developed in the 1970s [36]. The initial approach involved freezing platelets suspended in 6% dimethylsulfoxide (DMSO) with postthaw removal of the DMSO. Removing most of the DMSO before freezing and allowing omission of any postthaw processing were found to produce a comparable product [37] with obvious advantages in rapidly delivering the product to the patient with minimal equipment and training required in the hospital blood bank. These studies have been extensively reviewed [38]. A modification of this technique in which thawed platelets were resuspended in plasma rather than 0.9% saline was used by the Dutch military in Bosnia and Afghanistan and used by other groups [39,40■].

In-vitro and ex-vivo studies of CRYO-PLT show promising results. In a baboon model, 54% of thawed cryopreserved platelets were recovered 2 h after transfusion, more than was true for liquid platelets stored for 5 days [41]. CRYO-PLT have a higher capacity to bind Factor V [42] than liquid stored platelets, and produce more thromboxane A2 after ADP stimulation [37] (Table 1). In Phase I, human studies involving 32 healthy volunteers, CRYO-PLT obtained by apheresis and stored at less than -65°C were compared with fresh liquid-stored apheresis platelets. Radiolabelling allowed assessment of posttransfusion viability in 24 patients in whom CRYO-PLT showed a nearly 50% decrease in 24-h recovery, while the remaining circulating platelets persisted in the circulation for an almost normal lifespan [43]. More recently, a three-centre, dose-escalation, Phase I clinical trial in 28 bleeding (World Health Organization Bleeding Scale Grades II-IV), thrombocytopenic, haematology/oncology patients has been reported. In this study, no thrombotic events were identified and anecdotal experience of haemostatic bleeding response were presented, especially in patients with intracranial haemorrhage [71■].

In a study of trauma patients, 868 patients received 1679 CRYO-PLT units, apparently effectively and without adverse events [37]. Massively transfused trauma patients have benefited the most from early, aggressive treatment with CRYO-PLT. 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 CRYO-PLT to red blood cells (1:8) compared with a lower ratio was associated with an increased survival in the massive transfusion group (74 vs. 50%) [72]. The single controlled clinical trial of CRYO-PLT randomized 73 patients to cryopreserved or liquid-stored platelets if required for treatment of bleeding after cardiac surgery [73]. Blood loss in the 24 patients who received CRYO-PLT was significantly less than in the 29 patients who received liquid-stored platelets, despite lower posttransfusion platelet increments and a tendency towards decreased platelet survival. There was no observable difference in adverse events between the groups.

A postulated mechanism for greater haemostatic efficacy was that the cryopreservation/thawing process had 'preactivated' the platelets so that they bound more rapidly to the damaged endothelium following transfusion. CRYO-PLT produce more thromboxane A₂ and generate more procoagulant activity on their surface in response to stimulation which increases the ability of platelets to activate coagulation [37,42]. The formation of phosphatidyl-serine-exposed and tissue-factor expressing platelet microparticles is a significant contributor to the haemostatic effect of CRYO-PLT [74–76]. Thawed CRYO-PLT units contain a 15-fold higher concentration of functional platelet microparticles, as compared with fresh and Day 5 RT-PLT [74,76]. The microparticle containing supernatant of CRYO-PLT reduces clotting time and stimulates a twofold increase in phosphatidylserine and tissue factor induced peak thrombin generation compared with fresh platelet supernatant [74,76]. Interestingly, the clotting time of microparticle-filtered cryopreserved supernatant is similar to cryopreserved platelet concentrates, strongly suggesting that microparticles are the main mediator of the procoagulant activity of CRYO-PLT [75]. These findings are currently being tested in a prospective clinical trial in cardiac surgery patients in Australia [77] and are in preparation in United States.

CLINICAL CONSEQUENCES DERIVED OF STORAGE DEPENDENT PLATELET ACTIVATION

Multiple metabolic and signalling reactions that occur during the preparation and storage of platelet concentrates result in platelet activation and leads to accumulation of CD62P [78], sCD40L [79] and platelet microparticles (PMPs) [80], and activation of coagulation factors and generation of mediators of inflammation and immunity, which interact with vascular endothelial cells and neutrophils [81–83] (reviewed in [84]). Transfusion of platelet concentrates containing activated platelets and mediators of inflammation and cellular injury has been linked to platelet transfusion-associated adverse events. When the medical records of 15 237 hospitalized cancer patients who received platelet transfusions between 1995 and 2003 at 60 different medical centres were reviewed, they found that patients who received platelet transfusions had increased risks of venous and arterial thromboembolism, as well as death [85]. In addition, platelet transfusion had a negative effect on the survival of 433 adult patients undergoing first time orthotopic liver transplant [86]. In this study, increasing platelet transfusion was linked to worse survival in a dose-dependent fashion. Platelet transfusion has also been linked to coronary stent thrombosis in several studies. For example, three patients with gastrointestinal bleeding who received platelet transfusions early in the course of treatment following stenting were diagnosed with stent occlusion 6–17 h following platelet transfusion [87]. Similarly, a patient with aplastic anaemia received a platelet transfusion and subsequently developed a late stent thrombosis in a drug-eluting stent [88]. These studies emphasize the risk of platelet transfusion in patients with both early and late coronary stents for thrombotic occlusion. However, the retrospective, uncontrolled nature of these studies prevents the identification of the true risk of platelet transfusion associated thrombosis.

In fact, other studies failed to identify the same issue in patients undergoing cardiac surgery. For instance, in one such study, after adjusting for confounders, platelet transfusion was not

associated with increased risk of 30-day mortality or infective complications. Platelet transfusion was associated with higher rates of return to the operating room and interestingly, a decreased risk of thromboembolic events [89]. It is worth noting that these were retrospective studies using RT-PLT. In all cases, the nature and the severity of adverse events appear to be recipient specific and highly associated with patients with prothrombotic diseases. To illustrate this effect, platelet transfusions in patients with thrombotic thrombocytopenic purpura and heparin-induced thrombocytopenia, but not the patients with immune thrombocytopenia, appear to have higher odds of arterial thrombosis and mortality rate and these are considered contraindications for platelet transfusion [90]. In hospitalized cancer patients, platelet transfusion has been linked to a higher risk of venous thromboembolism and arterial thromboembolism as well as an increased in-hospital mortality rate [85]. Activation of neutrophils by platelet sCD40L accumulated during platelet storage contributes to transfusion-related acute lung injury [91], which appears to be responsible for increased postoperative mortality in patients receiving platelets during liver transplantation [92].

The finding that activated platelets and mediators of inflammation/immunity generated by platelets and other contaminating cells, namely leukocytes, are responsible for transfusion-related adverse events, suggests that prevention of platelet activation during the preparation and storage of platelet concentrates, and prestorage reduction of leukocytes may eliminate or diminish platelet transfusion-associated adverse events. In addition, discarding the storage medium and washing platelets prior to transfusion may also reduce the transfusion-associated adverse events [93,94].

CONCLUSION

Platelet storage at room temperature, as mandated by the FDA, comes with the inherent risk of microbial contamination and a limit of a 5-day shelf-life. With an accumulating understanding of the underlying molecular mechanisms discussed here that regulate the reaction of platelets to cold temperature stimulation and the irreversible changes that occur, a rationally designed refrigerated storage regimen is closer than ever to become a reality, which could resolve the platelet clearance problem and meet an urgent need in transfusion medicine. Although the developing pathogen reduction approach can be effective in reducing the risk of bacterial contamination and a cryopreservation regimen may benefit some trauma patients acutely, these approaches have their limits in dampening platelet activity or cannot support the haemostatic needs of cancer patients suffering from treatment-induced thrombocytopenia. In developing new platelet storage regimens that reduce the storage lesion, further improvement of the composition of current PAS to extend platelet metabolic and reactive life is also highly desirable. These platelet preservation strategies can complement current storage technologies and, together, they may achieve a more efficacious and safer protocol for longer term platelet storage.

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KEY POINTS

- Current safety and potency profiles of platelet products for human transfusion remain a source of concern due to bacterial contamination and the ‘platelet storage lesion’.
- Different methods of refrigerated/cryopreserved storage are under development, but there are significant concerns related to the level of activation of platelets with possible prothrombotic effects.
- Understanding the mechanisms involved in the platelet lesion and clearance is pivotal for developing targeted approaches destined to prevent the storage lesion and extend storage time.

Table 1.

Effects of storage on therapeutic platelet products stored at room temperature, cold or frozen when compared with platelets present in fresh platelet-rich plasma

In-vitro parameter	RT storage (5–7 days) [23–25]	Cold storage (5–7 days) [28–32,33,34,35]	DMSO frozen storage (months-years) [36–39,40,41–43]
Count of normal size platelets	Maintained	Decreased	Decreased
Mean platelet volume	Normal	Normal or slightly decreased	Decreased
pH	Very reduced	Mildly reduced	Mildly reduced
Ability to swirl	Maintained	Reduced	Reduced
Microparticle content	Low	Increased	Highly increased
Clot formation time	Increased	Maintained or decreased	Very decreased; normal when microparticles removed
Aggregation in response to ADP, collagen, epinephrine	Normal	Increased response	Decreased response
Thromboxane A2 production after ADP stimulation	Delayed	Normal or Increased	Reduced
Thrombin generation	Delayed Normal levels	Not delayed Increased levels	Not delayed Highly increased
vWF-GpIb binding	Normal	Increased	Increased
GpIb clustering	Not significantly increased	Highly increased	Not evaluated
Association state of 14–3–3c protein	Minimal	Increased	Increased
Lipid raft formation	Increased	Very increased	Very increased
Granule secretion	Mildly reduced	Increased	Highly increased
Cytoskeleton characteristics (electron microscopy)	Storage time dependent modest changes in canalicular system and granule content	Impaired, with increased pseudopodia and granule redistribution and reduced granule content. Altered microtubule and open canalicular system	Three populations which range from preserved but activated (increased pseudopodia and granule redistribution) to balloon-shaped with swollen canalicular system, reduced mitochondria, and fragmented, damaged spherical platelets with no preserved canalicular system, mitochondria or granules
Membrane CD62P expression	Increased	Very increased	Highly increased
Phosphatidylserine externalization	Modestly increased	Increased	Increased
Membrane CD40L	Similar to control	Increased	Increased
Factor V binding	Similar to control	Increased	Increased in microparticles
ATP production efficiency	Poor	Modestly reduced	Modestly reduced

RT, room temperature.