Platelet storage lesions: What more do we know now?

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Platelet storage lesions: what more do we know now?

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Abstract

Platelet concentrate (PC) transfusions are a life-saving adjunct to control and prevent bleeding in cancer, haematological, surgical and trauma patients. PC availability and safety are limited by the development of platelet storage lesions (PSLs) and risk of bacterial contamination. PSLs are a series of biochemical, structural and functional changes that occur from blood collection to transfusion. Understanding of PSLs is key for devising interventions that prolong PC shelf life to improve PC access and wastage. This paper will review advancements in clinical and mechanistic PSL research.

In brief, exposure to artificial surfaces and high centrifugation forces during PC preparation initiate PSLs by causing platelet activation, fragmentation and biochemical release. During room temperature storage, enhanced glycolysis and reduced mitochondrial function leads to glucose depletion, lactate accumulation and product acidification. Impaired adenosine triphosphate generation reduces platelet capacity to perform energetically demanding processes such as hypotonic stress responses and activation/aggregation. Storage-induced alterations in platelet surface proteins such as thrombin receptors and glycoproteins decrease platelet aggregation. During storage, there is an accumulation of immunoactive proteins such as leucocyte-derive cytokines (tumour necrosis factor α (TNF-α), interleukin (IL)-1α, IL-6, IL-8) and soluble CD40 ligand (sCD40L) which can participate in transfusion-related acute lung injury and non-haemolytic transfusion reactions. Storage-induced microparticles have been linked to enhanced platelet aggregation and immune system modulation.

Clinically, stored PCs have been correlated to reduced corrected count increment, post-transfusion platelet recovery and survival across multiple meta-analyses. Fresh PC transfusions have been associated with superior platelet function *in vivo*, however these differences were abrogated after a period of circulation. There is currently insufficient evidence to discern the effect of PSLs on transfusion safety.

Various bag and storage media changes have been proposed to reduce glycolysis and platelet activation during room temperature storage. Moreover, cryopreservation and cold storage have been proposed as potential methods to prolong PC shelf life by reducing platelet metabolism and bacterial proliferation. However, further work is required to elucidate and manage the PSLs specific to these storage protocols prior to its implementation in blood banks.

Keywords:

Platelets, platelet storage lesions, storage, age, transfusion, microparticles, transfusion reaction, cold-storage.

Abbreviations

5-HT=serotonin, ABLE=Age of Blood Evaluation, APC=activated protein C, ADP=adenosine diphosphate, ATP=adenosine triphosphate, BC=buffy coat, BTHC=*n*-Butyryl tri-*n*-hexyl citrate, CCI=corrected count increment, CD=cluster of differentiation, CD40L=CD40 ligand, CD62P=P-selectin, cGMP=cyclic guanine monophosphate, CI=confidence interval, DINCH=1,2-cyclohexane-dicarboxylic acid diisonoylester, DMSO=dimethyl sulfoxide, FDA= Food and Drug Administration, FVa=activated factor V, GP=glycoprotein, HSR=hypotonic stress responses, IL=interleukin, LPS=lipopolysaccharide, MCP-1=monocyte chemoattractant protein-1, NHTR=non-haemolytic transfusion reaction, NO=nitric oxide, PAR=protease-activated receptors, PAS=platelet additive solution, PC=platelet concentrate, PMPs=platelet microparticles, PRP=platelet rich plasma, PRT=pathogen reduction technologies, PS=phosphatidylserine, PSL=platelet storage PVC= plasticised polyvinyl chloride, ROTEM=rotational thromboelastometry, TCA=tricarboxylic acid, TEG=thromboelastography, TEHTM=tri-(ethylhexyl)-trimellitate, TNF-α=tumour necrosis factor α, TRALI=transfusion-related acute lung injury, TRANSFUSE= Standard Issue Transfusion versus Fresher Red Blood Cell Use in Intensive Care, TRIM=transfusion-related immunomodulation, USA=United States of America, VASP=vasodilator-stimulated phosphoprotein , vWF=von Willebrand factor

Introduction

Platelets are discoid, anucleate, cellular fragments that originate primarily from marrow megakaryocytes [1]. They circulate in the bloodstream for 8-10 days, after which they are cleared by macrophages. This occurs in the liver and spleen via recognition of absent, antibody-coated or desialylated surface glycoproteins (GP) [2-4].

Platelets play major roles in haemostasis, inflammation and wound healing [5]. Platelet adhesion to the subendothelial matrix in a damaged vessel is the initial step in primary haemostasis (Figure 1) [5]. Subsequently, platelets activate in the presence of biochemical agonists such as thrombin, adenosine diphosphate (ADP), thromboxane, epinephrine and serotonin (5-HT) [5]. Activated platelets then change shape to form extensive pseudopodia [5]. Dense and α granules fuse with the platelet plasma membrane via exocytosis to release granule contents [5]. ADP and 5-HT in dense granules amplify activation of surrounding platelets. α granules contain large adhesive proteins, mitogenic factors, coagulation factors and protease inhibitors that participate in platelet aggregation and coagulation (Table 1) [5]. P-selectin (CD62P) which resides in the α granule membrane at rest becomes exposed on the platelet plasma membrane, and plays key roles in platelet-leucocyte interactions.

Surface glycoproteins, GPIIb/IIIa and GPIb/V/IX complex on activated platelets interact with fibrinogen and von Willebrand factor (vWF) for platelet aggregation [5]. This process forms the haemostatic plug, signifying the end of primary haemostasis. Secondary haemostasis occurs when sufficient platelet activation initiates the coagulation cascade to generate thrombin and form a stable platelet-fibrin clot [5].

Platelet concentrate (PC) transfusions are a life-saving adjunct for the control and prevention of severe bleeding (Table 2). PC availability and safety are limited by the development of platelet storage lesions (PSLs) and risk of bacterial contamination. PSLs are the sum of biochemical and functional changes that occur in PCs from blood collection to transfusion. This paper will review advancements in clinical and mechanistic PSL research.

PC preparation and storage protocols

Platelets are purified from whole blood via centrifugation which separates blood components based on density. The three types of PC products— platelet rich plasma (PRP) PCs, buffy coat (BC) PCs and apheresis PCs; differ in the sequence of centrifugation steps [6] (Figure 2), described in detail by Greening *et al.* [6] and reviewed by Vassallo *et al.* [7]). Purified platelets are resuspended in plasma, platelet additive solution (PAS) or a mixture of both (reviewed in detail by Gulliksson [8] and Van der Meer [9]). Prior to storage, PCs are filtered based on size and charge to minimise contaminating proteins and leucocytes (United States of America (USA): $<5x10^6$ leucocytes/PC, other countries: $<1x10^6$ leucocytes/PC) [6]. In some jurisdictions, PCs undergo pathogen reduction technologies (PRTs) which inactivate viral and bacterial nucleic acid [10]. This prevents bacterial proliferation during storage and renders viruses ineffective thereby prolonging shelf life and preventing transfusion-induced infections.

Post-preparation, PCs are stored at 20-24°C under constant horizontal agitation in *n*-Butyryl tri-*n*-hexyl citrate (BTHC) or tri-(ethylhexyl)-trimellitate (TEHTM)

plasticised polyvinyl chloride (PVC) bags (reviewed in detail by Van der Meer and Korte [11]). PC shelf life is 3 days in Japan, 4 days in Germany, 5 days in Australia and USA and 7 days in the United Kingdom, Netherlands and Canada (see Supplementary Table 1 for jurisdictional differences) [12].

Mechanisms of PSL

PSLs occur due to platelet metabolism in the setting of *in vitro* preparation and storage pressures. These changes ultimately lead to disturbances in platelet activation, aggregation, coagulation and immune functions (Figure 3).

Pre-storage platelet activation

Platelet exposure to artificial surfaces and high centrifugation forces during PC preparation induce cellular activation, fragmentation and biochemical release [7, 13]. The extent of platelet activation varies between preparation methods. PRP PCs exhibit increased platelet activation markers (CD62P exposure, GPIIb/IIIa activation, loss of GPIb, platelet factor 4 (PF4) activity) compared to apheresis PCs as platelets are pelleted against the surface of the container during hard centrifugation [7]. In contrast, BC platelets are protected from the surface of the container by the red blood cell pellet during hard centrifugation resulting in the least centrifugation-induced activation [6]. Similar to its effects on pathogen nucleic acids, PRTs can inactivate platelet RNA to induce platelet activation [14]. Platelets from PRT-treated PCs were less responsive to collagen, ADP and thrombin-receptor activating peptide stimulation *in vitro* [15].

Storage-induced platelet activation

During storage, platelet-container interactions, room temperature environment and agitation are associated with platelet lysis and activation [11, 16, 17]. This is reflected by the progressive accumulation of lactate dehydrogenase, α granule (vWF, platelet derived growth factor) and dense granule (5-HT) contents in the storage media along with enhanced exposure of CD62P and phosphatidylserine (PS) over 5 days of storage [18-20]. While activated PCs may have improved haemostatic effects, increased CD62P exposure and GPIbα shedding are associated with decreased post-transfusion platelet survival [4, 21].

Alterations in ATP generation

Platelets are metabolically active and primarily rely on the hydrolysis of adenosine triphosphate (ATP) to drive the chemical reactions required for function and survival [22]. ATP is generated via two key pathways in platelets with 85% of ATP produced via the tricarboxylic acid (TCA) cycle (Figure 4) [23]. It follows that PC media undergo glucose depletion, lactate accumulation and acidification secondary to glycolysis with finite nutrients and absent clearance mechanisms [24]. Metabolomics analysis with ultra performance liquid chromatography demonstrated that glucose is exclusively converted to lactate via glycolysis, with decreased mitochondrial function secondary to TCA inhibition during the first 3 days of in vitro storage [25]. Towards, the end of shelf life, oxidative phosphorylation increases to generate ATP when substrates for glycolysis become depleted [25]. In spite of this, platelets from stored PCs exhibit inferior hypotonic stress responses (HSR) demonstrating a reduced capacity to perform energetically demanding processes [26]. HSR is associated with platelet survival *in vivo* and corroborates the finding that mitochondrial function is critical for platelet aggregation and survival [26, 27]. Importantly, mitochondrial

protection via acetyl-L-carnitine and ascorbic acid fail to improve platelet function suggesting that downstream non-bioenergetic processes are also impaired during storage [28].

Platelet surface receptor modifications

Storage-induced alterations in platelet surface proteins and metabolism lead to decreased platelet aggregation in response to thrombin, collagen, platelet activating factor, epinephrine and ADP [29-31]. Early studies found that storage-induced decreases in thrombin sensitivity were linked to reduced high affinity thrombin binding sites [32]. Thrombin binds G-protein-coupled protease-activated receptors 1 and 4 (PAR1, PAR4) to induce platelet secretion and aggregation. Both PAR1 and PAR4 expression decreased over 5 days of storage, impairing PAR4-mediated but not PAR1-mediated platelet aggregation [33]. Additionally, GPIbα shedding during PC storage is associated with decreased platelet aggregation [4]. The transfusion of stored PCs with 5G6 fragment, which blocks GPIbα shedding, shortened bleeding time in GPIbα-deficient mice [4]. Notably, GPIbα shedding is not universal across preparation methods as mean surface GPIbα expression remains stable until day 9 in BC PCs [34].

Nitric oxide pathway

PC storage also impairs platelet aggregation by enhancing multiple steps of the nitric oxide/cyclic nucleotide-dependent inhibitory pathway [35, 36]. Nitric oxide (NO) is a potent vasodilator and platelet inhibitor released by endothelial cells to maintain the balance of haemostasis. NO stimulation leads to intracellular cyclic guanine monophosphate (cGMP) accumulation which drives protein kinase G phosphorylation

of vasodilator-stimulated phosphoprotein (VASP). Phosphorylated VASP retains GPIIb/GPIIIa in resting conformation thereby inhibiting fibrinogen binding, adhesion and aggregation. Platelets in 5 day old PCs become sensitised to NO with stronger cGMP accumulation after NO donor stimulation [35]. Furthermore, basal cGMP concentration and VASP-phosphorylation rises by four to five times and 1.5 times respectively over 5 days of in vitro storage [36]. This leads to inhibited thrombin receptor activator-peptide-6 and collagen induced platelet aggregation in five day old PCs [35].

Thrombotic PMPs

PMPs are submicron membrane-bound vesicles that are shed during platelet activation, stress and integrin-mediated destabilisation of the actin cytoskeleton [37, 38]. PCs accumulate approximately 5000 PMPs/µl after 5 days of storage [39, 40]. Preparation- and storage-induced platelet microparticles (PMPs) amplify thrombosis via aggregation, coagulation and fibrinolysis processes. Storage-induced PMPs express PS, platelet surface proteins (GPIIb/IIIa, GPIb, cluster of differentiation (CD) 36, CD47) and coagulation factor proteins (tissue factor, activated factor V (FVa)) [38, 41]. PMPs initiate platelet plug formation via glycoprotein (GPIb, GPIIb/IIIa) adherence to collagen, vWF, fibrinogen and platelets at macrovascular and microvascular shear rates [42]. CD36 and CD47 work in concert to mediate platelet adherence to inflamed endothelium [43]. PMPs subsequently enhance the propagation phase of coagulation via thrombin generation. PMP surface PS assists the assembly of prothrombinase complexes that convert prothrombin to thrombin [42]. Additionally, PMPs contribute to thrombin-factor XI feedback pathway via membrane-dependent activation of factor XI – an important method of thrombin amplification at low tissue

factor concentrations [42]. PMP-associated thrombin generation is difficult to curb intravascularly as PMP FVa is resistant to activated protein C (APC) inactivation [39]. This has been tentatively attributed to poor APC access to FVa secondary to FVa binding to multimerin-1 and low-density lipoprotein receptor, along with FVa residence in lipid rafts [39]. Further research is required to demonstrate these processes.

Pro-inflammatory PSLs

In vivo, platelets interact with leucocytes via cytokine secretion and CD62P attachment to leucocyte surface proteins (Lewis x, sialyl lewis x, P-selectin glycoprotein ligand-1, soluble CD40 ligand (sCD40L)) [44]. These interactions are essential for immune system activation and modulation at sites of vascular injury and pathogen exposure [45]. Defects in these interactions have been attributed to non-haemolytic transfusion reactions (NHTRs), transfusion-related acute lung injury (TRALI) and transfusion-related immunomodulation (TRIM) (Figure 5) [45]. During PC preparation and storage, platelet and leucocyte activation lead to the accumulation of immunoactive proteins [46]. Early studies in PRP PCs found the accumulation of leucocyte-derived cytokines, tumour necrosis factor α (TNF-α), interleukin (IL)-1α, IL-6 and IL-8 during 5 day storage [47]. These chemokines were barely detectable in BC PCs after 5 day storage – likely secondary to superior leucocyte removal [48]. Leucoreduction can further decrease leucocyte count to $\leq 10^7$ leucocytes/PC and remove immunoactive proteins based on charge. This leads to low levels of leucocyte-derived cytokines that remain constant during storage [49]. Negatively charged filters are superior at removing positively charged proteins such as complement C4a, PF4, β-thromboglobulin and IL-8; but are associated with increased neutrophil, monocyte

and complement activation [50]. Despite leucoreduction, residual leucocytes retain the capacity to produce cytokines de novo during storage [49]. Moreover, leucoreduction have limited effect on platelet-derived immune factor and PMP accumulation [51, 52].

Stored PCs accumulate sCD40L which have been associated with immunological transfusion reactions such as TRALI and NHTRs via neutrophil activation and initiation of B-cell responses [45, 53, 54]. Storage-induced PMPs activate neutrophils to mediate leucocyte transmigration and enhance phagocytic activity [55]. In a two-event model of TRALI, neutrophils stimulated with sCD40L decreased lipopolysaccharide (LPS)-stimulated human microvascular endothelial cell viability. In this model, LPS simulated the pre-existing condition and sCD40L represented the transfusion-related insult [56]. The two-event model of stored PC supernatant-mediated TRALI is corroborated in an ovine model and prospective patient cohort [57, 58]. Additionally, PCs implicated in NHTRs have higher levels of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and sCD40L [59]. PCs with mediator levels in the highest quintiles did not induce NHTRs in >93% of patients suggesting that patient factors may play a substantial role in the pathogenesis of immunological adverse events [59].

Immunomodulatory PSLs

Storage-induced PMPs can modulate innate (monocyte, macrophage, dendritic cell) and adaptive immune cells (regulatory T cells) [41, 60, 61]. Together, these contribute to TRIM which has been associated with enhanced survival of renal allografts along with increased incidence of post-operative bacterial infections and short-term (up to

three-month) mortality [62]. Sadallah et al. observed that PMPs modified macrophage and dendritic cell differentiation towards less reactive states [41]. The affected cells exhibited reduced pro-inflammatory cytokine secretion (TNF-α, IL-10), impaired phagocytic activity and inhibited LPS-induced maturation [41]. Similar to TRALI, TRIM may occur via a two-event model whereby, LPS priming is required prior to stored PC supernatant exposure to reduce dendritic cell cytokine production [60]. More recently, PMPs have been demonstrated to prevent regulatory T cell differentiation into a pro-inflammatory state despite anti-CD3/CD28 antibody stimulation – suggesting that PMPs may have broader effects on the immune system than originally thought [61].

What do PSLs mean for clinical practice?

Laboratory studies have consistently associated PSLs with haemostatic and immune system dysfunction using *in vitro* and animal models. These results provide a strong benchtop argument that PC preparation and storage are linked with decreased transfusion efficacy and safety. However, do these findings translate into adverse patient outcomes? The efficacy and safety of stored PC transfusions have been predominantly assessed using small observational studies (Supplementary Table 2).

Stored PC efficacy

PC transfusion efficacy can be assessed using corrected count increment (CCI) along with post-transfusion platelet recovery and survival. On meta-analysis of 46 papers, Caram-Deelder *et al.* found that PCs stored for 0-2 days were superior to those stored for 5-7 days based on 1-hour and 24-hour CCIs with weighted mean differences of 2.68 (95% confidence interval (CI): 1.92-3.45) and 1.36 (95% CI: 0.08-2.63),

respectively [12]. Post-transfusion recovery and survival of PCs stored for five to seven days as a percentage of PCs stored for zero to two days were 80% and 71%, respectively [12]. This corroborates *in vitro* evidence that platelets in PCs undergo deteriorations in viability and apoptosis during storage [1]. Furthermore, stored platelets are selectively cleared from circulation due to CD62P exposure, GPIbα shedding and reduced membrane-associated sialic acid [4, 21, 63].

Importantly, CCIs and platelet survival do not indicate platelet function. Recent developments in bedside haemostatic testing have led to the use of viscoelastic tests such as thromboelastography (TEG) and rotational thromboelastometry (ROTEM) to assess the rate and strength of clot formation. TEG tests of blood collected from thrombocytopenic patients 1-hour post fresh BC PC transfusions demonstrated superior α angle (rate of clot formation) and K-time (time to fixed level of clot firmness) compared to those transfused with stored BC PC [64]. These differences were not statistically significant 24 hours post-transfusion suggesting that stored-induced platelet dysfunction can be recovered after a period of circulation [64]. In contrast, TEG of apheresis PCs demonstrate superior α angle, K-time and R-time (time to clot initiation) with prolonged storage [65]. This discrepancy could be attributed to differing preparation methods (Table 3); however PSLs can also vary significantly depending on the surrounding matrix. Early studies found that plasma addition to storage media is required to reduce PSLs [9]. Bikker *et al.* observed that post-transfusion platelet function recovery in thrombocytopenic patients can be recapitulated by incubating stored PCs with fresh whole blood [66].

CCIs, PC survival and recovery only provide cross-sectional data regarding transfusion efficacy. Furthermore, these measures require additional blood tests and/or the use of radioisotopes which can be expensive and logistically difficult to perform. Downstream outcomes such as time to transfusion and bleeding symptoms are cumulative indicators of platelet survival and function. A meta-analysis of four studies found that transfusion interval was 0.25 days (95% CI: 0 13–0 38) longer after ≤3 days old PC transfusion compared to ≥4 days old PC transfusion [67]. In blood stem cell transplant patients, reducing non-leucoreduced PC shelf life from five days to four days was associated with reduced bleeding symptoms such as epistaxis, haematuria and bleeding at multiple sites [68]. Transfusions of fresh PCs were linked to prolonged transfusion interval in acute myeloid leukaemia and intensive chemotherapy patients [69, 70]. Lastly, in multiple haematological malignancy cohorts, the requirement for a repeat PC transfusion within 24 hours increased with PC storage duration [71, 72]. Conversely, four studies found that platelet storage duration did not predict transfusion interval or time to first clinically significant bleed in haematology patients [73-76].

Stored PC safety

Studies of stored PC transfusion safety vary in reported outcomes and findings. A recent meta-analysis of seven studies associated increased PC storage duration with increased risks of immune-mediated transfusion reactions [67]. In 381 trauma patients, stored PC transfusions were associated with increased rates of sepsis but not ARDS, mortality, renal failure nor liver failure [77]. PC storage duration was not associated with 30-day mortality, prolonged hospital length of stay (>10 days), infectious complications or long-term survival (>5 years) in aortocoronary bypass

surgery patients [78]. There is insufficient evidence to discern the effect of PSLs on transfusion safety due to equivocal data in the setting of patient sample and outcome heterogeneity.

Most studies investigating the clinical effects of PC storage duration have utilised small cohorts, observational protocols and subset analyses which are vulnerable to bias and confounding factors. Large randomised controlled trials (RCTs) similar to the Age of Blood Evaluation (ABLE) and Standard Issue Transfusion versus Fresher Red Blood Cell Use in Intensive Care (TRANSFUSE) studies should be conducted to robustly assess the risks of stored PC transfusions [79]. This is particularly important considering the widespread implications of storage-induced adverse outcomes for clinical practice and blood bank management. For example, if fresh PCs could prolong transfusion interval in thrombocytopenic patients, its selective use for bleeding prophylaxis could significantly reduce exposure to alloimmunisation and standard transfusion risks.

PSL research: a moving target

Correlating clinical and laboratory findings

Platelets undergo activation and metabolic alterations during PC preparation and storage *in vitro*. However, *in vitro* functional defects correlate poorly with clinical observations. For example, platelet activation in transfused PCs was not correlated to post-operative chest drain fluid volume, a surrogate marker for blood loss after coronary bypass surgery [80]. Storage-induced defects in platelet aggregation *in vitro* can be overcome by using multiple agonists, which better approximate the *in vivo* environment [81]. It follows that PSLs interact with endogenous cells and mediators

in a complex manner to affect multiple targets of the haemostatic cascade. While simple *in vitro* models have advanced knowledge of individual PSL components, complex models that incorporate dynamic factors are required to understand how PSLs interact with recipient conditions. Future studies could test PSLs using vascular (under flow conditions) and transfusion (mixed with whole blood) models that better approximate the *in vivo* environment.

Complex models and *in vivo* studies are complicated by numerous sources of variability. For example, leucocyte concentration and platelet activation can vary between donors (Supplementary Table 3) [21, 50, 82-84]. Although there has been some work investigating the merits of donor selection to improve PC quality and safety, currently there is insufficient evidence to select donors based on these traits [82]. Considering that the myriad of interactions between PC and recipient factors are poorly understood, *in vivo* studies are vulnerable to unchecked confounders which could mask small positive effects that are limited to selective patient populations. Similarly, meta-analyses are difficult to interpret due to jurisdictional and time-associated variations in donor characteristics, PC preparation methods and shelf life. This could be addressed by combining patient level data from studies with the same storage guidelines. However, the merits of intensive clinical data mining to identify such a small selective adverse effect margin has to be questioned in the context of an already short PC shelf life of 5-7 days with little room to move should an adverse effect be found. This is particularly significant considering that median PC expiry rates decrease from 6.8% to 3.1% when moving from 5 to 7 day jurisdictions [85]. Instead, research should focus on the development of technologies that abrogate PSLs and prolong shelf life.

Improving room temperature platelet concentrates

PSLs secondary to platelet metabolism and activation at room temperature storage can be improved via advances in bag materials and storage media (Figure 3). Bag materials such as 1,2-cyclohexane-dicarboxylic acid diisonoylester (DINCH)-PVC are purported to be less toxic than current BTHC- or TEHTM- PVC bags, owing to decreased phthalate contamination [85, 86]. Furthermore, PCs stored in DINCH-PVC for 7 days have lower glycolytic activity and platelet activation compared to BTHC-PVC [86]. Transfusion studies of DINCH-PVC stored PCs are required prior to their implementation in blood banks. Several investigators have used the addition of small molecules to combat specific PSL mechanisms (Supplementary Table 4). L-carnitine facilitates transport of long chain fatty acids into the mitochondrial matrix to redirect ATP generation towards β-oxidation thereby, reducing glucose consumption and lactate accumulation in PRP PCs [87]. Mitogen activated protein kinase inhibitors such as VX-702, have been added to PAS to prevent GPIb shedding and oxidative damage [88]. VX-702 treated PCs exhibit improved glucose availability, pH, morphology score, HSR, reactive oxygen levels and platelet activation [88].

Cryopreserved platelet concentrates

Decreasing storage temperature can lengthen PC shelf life by reducing metabolism-related PSLs and bacterial contamination. The most commonly used PC cryopreservation protocol involve adding dimethyl sulfoxide (DMSO) to room temperature PCs (final concentration of 4-6%), followed by centrifugation to remove DMSO prior to storage at -80°C for 2-4 years [89]. Cryopreserved PCs are placed in

a water bath for rapid thawing and then reconstituted in: plasma, PAS or a mixture of both similar to room temperature PCs [89]. These PCs have mean survival of 7.0 days and 24 hour recovery rates of 41.6%, which satisfies the USA Food and Drug Administration (FDA) requirements for survival but not 24 hour recovery [90]. Cryopreserved PCs demonstrate increased activation via P-selectin expression along with decreased aggregation responses to ADP and collagen [91]. Despite decreased *in vitro* platelet aggregation efficacy, cardiopulmonary bypass patients receiving cryopreserved PCs experienced decreased blood loss and post-operative blood production requirements compared to those receiving room temperature PCs [92]. This is supported by anecdotal evidence from Dutch military hospitals whereby, cryopreserved PCs stopped severe bleeding without increasing platelet count, suggesting that soluble components such as microparticles may contribute to the procoagulant effects of cryopreserved PCs [93]. To date, cryopreserved PCs are approved for general use in France and only for military operations in the Netherlands and Germany [89]. The CLIP trial (ACTRN12612001261808) is a multi-centre blinded RCT currently underway to compare the efficacy of cryopreserved PCs versus standard PCs for the management of post-surgical bleeding in civilian populations [94]. Similarly, large RCTs are required to determine the efficacy of prophylactic cryopreserved PC transfusion. Even if the efficacy of cryopreserved PCs is established in civilian settings, the extra time and equipment costs associated with PC cryopreservation and thawing remain a major barrier for widespread use. Additionally, standardisation of cryopreserved PC preparation and thawing are required prior to implementation.

Cold stored platelet concentrates

Alternatively, cold stored (at 2-6°C) PCs are easier to prepare and store. In 2015, the FDA approved cold stored PCs for resuscitation of bleeding patients. Cold stored PCs have decreased bacterial contamination risks and improved haemostasis compared to room temperature PCs [95, 96]. Compared to room temperature PCs, cold stored PC transfusions led to superior platelet function in paediatric cardiac surgery patients and shorter bleeding times in adults taking aspirin[97, 98]. A clinical trial is currently underway at Haukeland University Hospital, Norway to investigate the efficacy of cold stored PCs in cardiothoracic surgery patients (NCT02495506). Notably, cold stored PCs exhibit decreased survival and recovery compared to room temperature PCs making them less suitable for bleeding prophylaxis in thrombocytopenic patients [99].

Platelet surface glycoprotein alterations and cold-induced platelet activation are associated with reduced post-transfusion platelet survival (Figure 6). During cold-storage, platelets undergo Rho GTPase-induced clustering of β-*N*-acetylglucosamine on α subunits of GPIb [100, 101]. These microdomains are recognised by complement receptors on macrophages leading to platelet phagocytosis and clearance [100]. Enzymatic galactosylation prevented complement receptor recognition of β-*N*-acetylglucosamine residues *in vitro* [102]. However, enzymatic galactosylation did not improve platelet survival *in vivo*, suggesting that multiple mechanisms may be involved in cold-stored platelet clearance [99]. RhoA inhibitors addition to storage media work upstream to prevent glycoprotein clustering during cold storage. Such PCs led to similar collagen-induced platelet shape change and aggregation, and bleeding time correction in aspirated mice and Rhesus monkeys compared to room temperature stored platelet concentrates [103].

Cold-induced platelet activation causes calcium influx leading to calcium-dependent gelosin activation, phosphoinositide-mediated actin polymerisation and ultimately, loss of platelet discoid morphology [1]. Enhanced intracellular calcium also facilitates α-granule and lysosomal content release; thereby, amplifying platelet activation during cold storage [101]. Attempts have been made to curtail cold-induced platelet activation via metabolic suppression with glucose-free media and antimycin A (mitochrondrial inhibitor) [101]. Double blockade was required to decrease cold-induced platelet activation and macrophage binding [104]. Notably, antimycin A is highly toxic at its effective concentration and inappropriate for clinical use. Storage media with second messenger effectors (ThromboSol: LifeCell Corporation) such as amiloride, adenosine, sodium nitroprusside, dipyridamole, ticlopidine and quinacrine was formulated to prevent cold-induced platelet activation [105]. Amiloride inhibited sodium/hydrogen ion pump, adenosine inhibited intracellular cyclic adenosine monophosphate and sodium nitroprusside enhanced nitric oxide release [106]. PCs cold stored in ThromboSol showed reduced platelet activation and improved *in vitro* function compared to untreated PCs however, its impact on platelet survival *in vivo* remains to be seen [106, 107]. More recently, resolvins, a class of specialised proresolving mediators, have been proposed as a storage media additive to reduce cold-induced platelet activation [108]. Further investigations are required to link reduced platelet activation to improved post-transfusion platelet survival.

It follows that PSLs are storage method dependent – necessitating the shift towards investigating cold stored PSLs. Understanding PSLs as a function of storage method

are necessary to facilitate cold storage, which can prolong shelf life and simplify PC storage protocols.

Turning the tables on microparticles

For example, while PSLs such as PMPs can lead to enhanced thrombogenicity and immunoactivity, microparticles can also transfer peptides, proteins, lipids, microRNA, mRNA, and DNA from one cell to another to alter recipient cell function [109]. Recent advances in microfluidics have enabled the development of liposomes less than 30μm loaded with desired transmembrane and cytoskeletal proteins [110]. In the future, these bioengineered microparticles could be incorporated in storage media to prevent or replenish storage-related changes in platelet membrane markers such as CD62P, PAR1, PAR4 and GPIbα. Furthermore, the divergence between microparticle and parent surface markers suggest that microparticle formation remove specific proteins from the cell membrane [109]. Selective vesiculation may be applied to remove deleterious markers from stored platelets. In this way, identification of PSLs are critical for devising methods to abrogate PSLs and prolong PC shelf life.

Final remarks

PSLs develop during PC preparation and storage. Mechanical and biochemical forces during PC preparation induce platelet activation which persist throughout storage duration. Changes in platelet surface receptors and PMP elaboration impair platelet aggregation. Soluble factors and PMPs accumulated during storage interfere with the delicate balance of inflammatory and immunomodulatory factors. Clinically, PSLs have been associated with reduced CCI and platelet survival but not adverse transfusion events. Current developments to reduce room temperature PSLs have

focused on reducing platelet glycolysis and activation, via bag material and storage media changes. Cryopreservation and cold storage offer the potential for prolonged storage, reduced bacterial proliferation and improved haemostasis in trauma populations. Further research is required to define storage-specific PSLs so that the mechanisms underlying cold-induced platelet activation and reduced platelet survival can be addressed.

Declarations of interest

None.

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References

[1] M. Shrivastava. The platelet storage lesion, *Transfus Apher Sci* 2009; 41:105-113.

[2] S.K. Dasgupta, E.R. Argaiz, J.E. Mercado, H.O. Maul, J. Garza, A.B. Enriquez, H. Abdel-Monem, A. Prakasam, M. Andreeff, P. Thiagarajan. Platelet senescence and phosphatidylserine exposure, *Transfusion* 2010; 50:2167-2175.

[3] R. Grozovsky, K.M. Hoffmeister, H. Falet. Novel clearance mechanisms of platelets, *Curr Opin Hematol* 2010; 17:585-589.

[4] W. Chen, X. Liang, A.K. Syed, P. Jessup, W.R. Church, J. Ware, C.D. Josephson, R. Li. Inhibiting GPIbalpha Shedding Preserves Post-Transfusion Recovery and Hemostatic Function of Platelets After Prolonged Storage, *Arterioscler Thromb Vasc Biol* 2016; 36:1821-1828.

[5] K. Jurk, B.E. Kehrel. Platelets: physiology and biochemistry, *Semin Thromb Hemost* 2005; 31:381-392.

[6] D.W. Greening, R.L. Sparrow, R.J. Simpson. Preparation of platelet concentrates, *Methods Mol Biol* 2011; 728:267-278.

[7] R.R. Vassallo, S. Murphy. A critical comparison of platelet preparation methods, *Curr. Opin. Hematol.* 2006; 13:323-330.

[8] H. Gulliksson. Platelet storage media, *Vox Sang* 2014; 107:205-212.

[9] P.F. van der Meer. PAS or plasma for storage of platelets? A concise review, *Transfus Med* 2016; 26:339-342.

[10] J. Seghatchian, T. Hervig, J.S. Putter. Effect of pathogen inactivation on the storage lesion in red cells and platelet concentrates, *Transfus Apher Sci* 2011; 45:75-84.

[11] P.F. van der Meer, D. de Korte. Platelet preservation: agitation and containers, *Transfus Apher Sci* 2011; 44:297-304.

[12] C. Caram-Deelder, A.L. Kreuger, J. Jacobse, J.G. van der Bom, R.A. Middelburg. Effect of platelet storage time on platelet measurements: a systematic review and meta-analyses, *Vox Sang* 2016.

[13] M. Rijkers, F.P. van Alphen, P.F. van der Meer, D. de Korte, F.W.G. Leebeek, A.B. Meijer, J. Voorberg, G.A. Jansen. Label Free Quantitative Mass Spectrometry Identifies Processes Linked to Platelet Degranulation As Early Events during Platelet Storage, *Blood* 2016; 128:2638-2638.

[14] A. Osman, W.E. Hitzler, P. Provost. The platelets' perspective to pathogen reduction technologies, *Platelets* 2017:1-8.

[15] A. Osman, W.E. Hitzler, C.U. Meyer, P. Landry, A. Corduan, B. Laffont, E. Boilard, P. Hellstern, E.C. Vamvakas, P. Provost. Effects of pathogen reduction systems on platelet microRNAs, mRNAs, activation, and function, *Platelets* 2015; 26:154-163.

[16] E.L. Snyder, A. Hezzey, A.J. Katz, J. Bock. Occurrence of the release reaction during preparation and storage of platelet concentrates, *Vox Sang* 1981; 41:172-177.

[17] E. Maurer-Spurej, G. Pfeiler, N. Maurer, H. Lindner, O. Glatter, D.V. Devine. Room temperature activates human blood platelets, *Lab Invest* 2001; 81:581-592.

[18] M.T. Aye, D.S. Palmer, A. Giulivi, S. Hashemi. Effect of filtration of platelet concentrates on the accumulation of cytokines and platelet release factors during storage, *Transfusion* 1995; 35:117-124.

[19] A.M. Albanyan, P. Harrison, M.F. Murphy. Markers of platelet activation and apoptosis during storage of apheresis- and buffy coat-derived platelet concentrates for 7 days, *Transfusion* 2009; 49:108-117.

[20] S. Plenchette, M. Moutet, M. Benguella, J.P. N'Gondara, F. Guigner, C. Coffe, L. Corcos, A. Bettaieb, E. Solary. Early increase in DcR2 expression and late activation of caspases in the platelet storage lesion, *Leukemia* 2001; 15:1572-1581.

[21] H.M. Rinder, M. Murphy, J.G. Mitchell, J. Stocks, K.A. Ault, R.S. Hillman. Progressive platelet activation with storage: evidence for shortened survival of activated platelets after transfusion, *Transfusion* 1991; 31:409-414.

[22] N. Tynngard. Preparation, storage and quality control of platelet concentrates, *Transfus Apher Sci* 2009; 41:97-104.

[23] H. Kilkson, S. Holme, S. Murphy. Platelet metabolism during storage of platelet concentrates at 22 degrees C, *Blood* 1984; 64:406-414.

[24] S.J. Slichter. Preservation of platelet viability and function during storage of concentrates, *Prog Clin Biol Res* 1978; 28:83-100.

[25] G. Paglia, O.E. Sigurjonsson, O. Rolfsson, S. Valgeirsdottir, M.B. Hansen, S. Brynjolfsson, S. Gudmundsson, B.O. Palsson. Comprehensive metabolomic study of platelets reveals the expression of discrete metabolic phenotypes during storage, *Transfusion* 2014; 54:2911-2923.

[26] S. Ravi, B. Chacko, P.A. Kramer, H. Sawada, M.S. Johnson, D. Zhi, M.B. Marques, V.M. Darley-Usmar. Defining the effects of storage on platelet bioenergetics: The role of increased proton leak, *Biochim Biophys Acta* 2015; 1852:2525-2534.

[27] J.P. Perales Villarroel, R. Figueredo, Y. Guan, M. Tomaiuolo, M.A. Karamercan, J. Welsh, M.A. Selak, L.B. Becker, C. Sims. Increased platelet storage time is associated with mitochondrial dysfunction and impaired platelet function, *J Surg Res* 2013; 184:422-429.

[28] T. Hayashi, S. Tanaka, Y. Hori, F. Hirayama, E.F. Sato, M. Inoue. Role of mitochondria in the maintenance of platelet function during in vitro storage, *Transfus Med* 2011; 21:166-174.

[29] S.D. Shukla, W.J. Morrison, D.M. Klachko. Response to platelet-activating factor in human platelets stored and aged in plasma. Decrease in aggregation, phosphoinositide turnover, and receptor affinity, *Transfusion* 1989; 29:528-533.

[30] N.J. Scott, J.R. Harris, A.E. Bolton. Effect of storage on platelet release and aggregation responses, *Vox Sang* 1983; 45:359-366.

[31] M. Owens, S. Holme, A. Heaton, S. Sawyer, S. Cardinali. Post-transfusion recovery of function of 5-day stored platelet concentrates, *Br J Haematol* 1992; 80:539-544.

[32] M.L. Lozano, J. Rivera, R. Gonzalez-Conejero, J.M. Moraleda, V. Vicente. Loss of high-affinity thrombin receptors during platelet concentrate storage impairs the reactivity of platelets to thrombin, *Transfusion* 1997; 37:368-375.

[33] A. Schlagenhauf, N. Kozma, B. Leschnik, T. Wagner, W. Muntean. Thrombin receptor levels in platelet concentrates during storage and their impact on platelet functionality, *Transfusion* 2012; 52:1253-1259.

[34] M. Rijkers, P.F. van der Meer, I.J. Bontekoe, B.B. Daal, D. de Korte, F.W. Leebeek, J. Voorberg, A.J. Jansen. Evaluation of the role of the GPIb-IX-V receptor complex in development of the platelet storage lesion, *Vox Sang* 2016; 111:247-256.

[35] A. Kobsar, E. Klinker, S. Kuhn, A. Koessler, P. Yilmaz, M. Boeck, J. Koessler. Increasing susceptibility of nitric oxide-mediated inhibitory platelet signaling during storage of apheresis-derived platelet concentrates, *Transfusion* 2014; 54:1782-1789.

[36] A. Kobsar, E. Putz, P. Yilmaz, E. Weinig, M. Boeck, J. Koessler. Decreasing phosphodiesterase 5A activity contributes to platelet cGMP accumulation during storage of apheresis-derived platelet concentrates, *Transfusion* 2014; 54:1008-1014.

[37] S. Cauwenberghs, M.A. Feijge, A.G. Harper, S.O. Sage, J. Curvers, J.W. Heemskerk. Shedding of procoagulant microparticles from unstimulated platelets by integrin-mediated destabilization of actin cytoskeleton, *FEBS Lett* 2006; 580:5313-5320.

[38] A.P. Bode, S. Holme, W.A. Heaton, M.S. Swanson. Extended storage of platelets in an artificial medium with the platelet activation inhibitors prostaglandin E1 and theophylline, *Vox Sang* 1991; 60:105-112.

[39] E.J. Magdeleyns, J.F. Keuren, J. Curvers. Factor Va, bound to microparticles released during platelet storage, is resistant to inactivation by activated protein C, *Transfusion* 2007; 47:1880-1888.

[40] J.F. Keuren, E.J. Magdeleyns, J.W. Govers-Riemslag, T. Lindhout, J. Curvers. Effects of storage-induced platelet microparticles on the initiation and propagation phase of blood coagulation, *Br J Haematol* 2006; 134:307-313.

[41] S. Sadallah, C. Eken, P.J. Martin, J.A. Schifferli. Microparticles (ectosomes) shed by stored human platelets downregulate macrophages and modify the development of dendritic cells, *J Immunol* 2011; 186:6543-6552.

[42] J.F. Keuren, E.J. Magdeleyns, A. Bennaghmouch, E.M. Bevers, J. Curvers, T. Lindhout. Microparticles adhere to collagen type I, fibrinogen, von Willebrand factor and surface immobilised platelets at physiological shear rates, *Br J Haematol* 2007; 138:527-533.

[43] P. Lagadec, O. Dejoux, M. Ticchioni, F. Cottrez, M. Johansen, E.J. Brown, A. Bernard. Involvement of a CD47-dependent pathway in platelet adhesion on inflamed vascular endothelium under flow, *Blood* 2003; 101:4836-4843.

[44] C. Cerletti, G. de Gaetano, R. Lorenzet. Platelet - leukocyte interactions: multiple links between inflammation, blood coagulation and vascular risk, *Mediterr J Hematol Infect Dis* 2010; 2:e2010023.

[45] C.N. Morrell, A.A. Aggrey, L.M. Chapman, K.L. Modjeski. Emerging roles for platelets as immune and inflammatory cells, *Blood* 2014; 123:2759-2767.

[46] F. Boehlen, K.J. Clemetson. Platelet chemokines and their receptors: what is their relevance to platelet storage and transfusion practice?, *Transfus Med* 2001; 11:403-417.

[47] G. Stack, E.L. Snyder. Cytokine generation in stored platelet concentrates, *Transfusion* 1994; 34:20-25.

[48] W.A. Flegel, M. Wiesneth, D. Stampe, K. Koerner. Low cytokine contamination in buffy coat-derived platelet concentrates without filtration, *Transfusion* 1995; 35:917-920.

[49] D. Hartwig, C. Hartel, H. Hennig, M. Muller-Steinhardt, P. Schlenke, H. Kluter. Evidence for de novo synthesis of cytokines and chemokines in platelet concentrates, *Vox Sang* 2002; 82:182-190.

[50] J. Seghatchian. Platelet storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers, *Transfus Apher Sci* 2006; 34:125-130.

[51] F. Cognasse, F. Boussoulade, P. Chavarin, S. Acquart, P. Fabrigli, B. Lamy, O. Garraud. Release of potential immunomodulatory factors during platelet storage, *Transfusion* 2006; 46:1184-1189.

[52] K.E. Nollet, S. Saito, T. Ono, A. Ngoma, H. Ohto. Microparticle formation in apheresis platelets is not affected by three leukoreduction filters, *Transfusion* 2013; 53:2293-2298.

[53] J. Sahler, K. Grimshaw, S.L. Spinelli, M.A. Refaai, R.P. Phipps, N. Blumberg. Platelet storage and transfusions: new concerns associated with an old therapy, *Drug Discov Today Dis Mech* 2011; 8:e9-e14.

[54] P. Vanichakarn, P. Blair, C. Wu, J.E. Freedman, S. Chakrabarti. Neutrophil CD40 enhances platelet-mediated inflammation, *Thromb Res* 2008; 122:346-358.

[55] W. Jy, W.W. Mao, L. Horstman, J. Tao, Y.S. Ahn. Platelet microparticles bind, activate and aggregate neutrophils in vitro, *Blood Cells. Mol. Dis.* 1995; 21:217-231; discussion 231a.

[56] S.Y. Khan, M.R. Kelher, J.M. Heal, N. Blumberg, L.K. Boshkov, R. Phipps, K.F. Gettings, N.J. McLaughlin, C.C. Silliman. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury, *Blood* 2006; 108:2455-2462.

[57] J.P. Tung, Y.L. Fung, M. Nataatmadja, K.I. Colebourne, H.M. Esmaeel, K. Wilson, A.G. Barnett, P. Wood, C.C. Silliman, J.F. Fraser. A novel in vivo ovine model of transfusion-related acute lung injury (TRALI), *Vox Sang* 2011; 100:219-230.

[58] C.C. Silliman, L.K. Boshkov, Z. Mehdizadehkashi, D.J. Elzi, W.O. Dickey, L. Podlosky, G. Clarke, D.R. Ambruso. Transfusion-related acute lung injury: epidemiology and a prospective analysis of etiologic factors, *Blood* 2003; 101:454-462.

[59] N. Blumberg, K.F. Gettings, C. Turner, J.M. Heal, R.P. Phipps. An association of soluble CD40 ligand (CD154) with adverse reactions to platelet transfusions, *Transfusion* 2006; 46:1813-1821.

[60] A.J. Perros, A.M. Christensen, R.L. Flower, M.M. Dean. Soluble Mediators in Platelet Concentrates Modulate Dendritic Cell Inflammatory Responses in an Experimental Model of Transfusion, *J Interferon Cytokine Res* 2015; 35:821-830.

[61] S. Dinkla, B. van Cranenbroek, W.A. van der Heijden, X. He, R. Wallbrecher, I.E. Dumitriu, A.J. van der Ven, G.J. Bosman, H.J. Koenen, I. Joosten. Platelet microparticles inhibit IL-17 production by regulatory T cells through P-selectin, *Blood* 2016; 127:1976-1986.

[62] E.C. Vamvakas, M.A. Blajchman. Transfusion-related immunomodulation (TRIM): an update, *Blood Rev.* 2007; 21:327-348.

[63] G. Soslau, J. Giles. The loss of sialic acid and its prevention in stored human platelets, *Thromb Res* 1982; 26:443-455.

[64] W.W. Roeloffzen, H.C. Kluin-Nelemans, N.J. Veeger, L. Bosman, J.T. de Wolf. Transfused stored platelets have the same haemostatic function as circulating native platelets, *Vox Sang.* 2010; 99:123-130.

[65] I.J. Bontekoe, P.F. van der Meer, D. de Korte. Determination of thromboelastographic responsiveness in stored single-donor platelet concentrates, *Transfusion* 2014; 54:1610-1618.

[66] A. Bikker, E. Bouman, S. Sebastian, S.J. Korporaal, R.T. Urbanus, R. Fijnheer, L.A. Boven, M. Roest. Functional recovery of stored platelets after transfusion, *Transfusion* 2016; 56:1030-1037.

[67] A.L. Kreuger, C. Caram-Deelder, J. Jacobse, J.L. Kerkhoffs, J.G. van der Bom, R.A. Middelburg. Effect of storage time of platelet products on clinical outcomes after transfusion: a systematic review and meta-analyses, *Vox Sang* 2017.

[68] H.G. Heuft, L. Goudeva, J. Krauter, D. Peest, S. Buchholz, A. Tiede. Effects of platelet concentrate storage time reduction in patients after blood stem cell transplantation, *Vox Sang* 2013; 105:18-27.

[69] S.J. Slichter, K. Davis, H. Enright, H. Braine, T. Gernsheimer, K.J. Kao, T. Kickler, E. Lee, J. McFarland, J. McCullough, G. Rodey, C.A. Schiffer, R. Woodson. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients, *Blood* 2005; 105:4106-4114.

[70] C.A. Akkok, L. Brinch, G.F. Lauritzsen, B.G. Solheim, J. Kjeldsen-Kragh. Clinical effect of buffy-coat vs. apheresis platelet concentrates in patients with severe thrombocytopenia after intensive chemotherapy, *Vox Sang* 2007; 93:42-48.

[71] J.K. Duguid, R. Carr, J.A. Jenkins, J.L. Hutton, G.F. Lucas, J.M. Davies. Clinical evaluation of the effects of storage time and irradiation on transfused platelets, *Vox Sang* 1991; 60:151-154.

[72] F. Norol, M. Kuentz, C. Cordonnier, F. Beaujean, C. Haioun, J.P. Vernant, N. Duedari. Influence of clinical status on the efficiency of stored platelet transfusion, *Br J Haematol* 1994; 86:125-129.

[73] D.J. Triulzi, S.F. Assmann, R.G. Strauss, P.M. Ness, J.R. Hess, R.M. Kaufman, S. Granger, S.J. Slichter. The impact of platelet transfusion characteristics on posttransfusion platelet increments and clinical bleeding in patients with hypoproliferative thrombocytopenia, *Blood* 2012; 119:5553-5562.

[74] S. MacLennan, K. Harding, C. Llewelyn, L. Choo, L. Bakrania, E. Massey, S. Stanworth, K. Pendry, L.M. Williamson. A randomized noninferiority crossover trial of corrected count increments and bleeding in thrombocytopenic hematology patients receiving 2- to 5- versus 6- or 7-day-stored platelets, *Transfusion* 2015; 55:1856-1865; quiz 1855.

[75] M.F. Leach, J.P. AuBuchon. Effect of storage time on clinical efficacy of single-donor platelet units, *Transfusion* 1993; 33:661-664.

[76] J.L. Kerkhoffs, J.C. Eikenboom, M.S. Schipperus, R.J. van Wordragen-Vlaswinkel, R. Brand, M.S. Harvey, R.R. de Vries, R. Barge, D.J. van Rhenen, A. Brand. A multicenter randomized study of the efficacy of transfusions with platelets stored in platelet additive solution II versus plasma, *Blood* 2006; 108:3210-3215.

[77] K. Inaba, B.C. Branco, P. Rhee, L.H. Blackbourne, J.B. Holcomb, P.C. Spinella, I. Shulman, J. Nelson, D. Demetriades. Impact of the duration of platelet storage in critically ill trauma patients, *J Trauma* 2011; 71:1766-1773; discussion 1773-1764.

[78] I.J. Welsby, E. Lockhart, B. Phillips-Bute, M.L. Campbell, J.P. Mathew, M.F. Newman, E. Peterson, C.A. Milano. Storage age of transfused platelets and outcomes after cardiac surgery, *Transfusion* 2010; 50:2311-2317.

[79] M.S. Ng, A.S. Ng, J. Chan, J.P. Tung, J.F. Fraser. Effects of packed red blood cell storage duration on post-transfusion clinical outcomes: a meta-analysis and systematic review, *Intensive Care Med.* 2015; 41:2087-2097.

[80] M.J. Dijkstra-Tiekstra, R.N. Pietersz, P.C. Huijgens. Correlation between the extent of platelet activation in platelet concentrates and in vitro and in vivo parameters, *Vox Sang* 2004; 87:257-263.

[81] S. Shapira, Z. Friedman, H. Shapiro, K. Presseizen, J. Radnay, M.H. Ellis. The effect of storage on the expression of platelet membrane phosphatidylserine and the

subsequent impacton the coagulant function of stored platelets, *Transfusion* 2000; 40:1257-1263.

[82] A.M. Kelly, S.F. Garner, T. Foukaneli, T.R. Godec, N. Herbert, B.C. Kahan, A. Deary, L. Bakrania, C. Llewelyn, W.H. Ouwehand, L.M. Williamson, R.A. Cardigan. The effect of variation in donor platelet function on transfusion outcome: a semirandomized controlled trial, *Blood* 2017; 130:214-220.

[83] M. Dzieciatkowska, A. D'Alessandro, T.A. Burke, M.R. Kelher, E.E. Moore, A. Banerjee, C.C. Silliman, B.F. West, K.C. Hansen. Proteomics of apheresis platelet supernatants during routine storage: Gender-related differences, *J Proteomics* 2015; 112:190-209.

[84] J.C. Zimring, S. Slichter, K. Odem-Davis, J.R. Felcyn, L.M. Kapp, L.N. Bell, P.R. Gunst, J. Corson, M.K. Jones, E. Pellham, S.L. Bailey, X. Fu. Metabolites in stored platelets associated with platelet recoveries and survivals, *Transfusion* 2016; 56:1974-1983.

[85] M.H. Yazer, S. Abraham, N. Beckman, G. Folléa. International Society for Blood Transfusion international survey on blood product wastage in hospitals, *ISBT Science Series* 2016; 11:24-31.

[86] J.W. Lagerberg, E. Gouwerok, R. Vlaar, M. Go, D. de Korte. In vitro evaluation of the quality of blood products collected and stored in systems completely free of di(2-ethylhexyl)phthalate-plasticized materials, *Transfusion* 2015; 55:522-531.

[87] M.R. Deyhim, S.A. Mesbah-Namin, F. Yari, M. Taghikhani, N. Amirizadeh. L-carnitine effectively improves the metabolism and quality of platelet concentrates during storage, *Ann Hematol* 2014.

[88] A. Skripchenko, H. Awatefe, D. Thompson-Montgomery, A. Myrup, A. Turgeon, S.J. Wagner. An inhibition of p38 mitogen activated protein kinase delays the platelet storage lesion, *PloS one* 2013; 8:e70732.

[89] L.J. Dumont, S.J. Slichter, M.C. Reade. Cryopreserved platelets: frozen in a logjam?, *Transfusion* 2014; 54:1907-1910.

[90] L.J. Dumont, J.A. Cancelas, D.F. Dumont, A.H. Siegel, Z.M. Szczepiorkowski, N. Rugg, P.G. Pratt, D.N. Worsham, E.L. Hartman, S.K. Dunn, M. O'Leary, J.H. Ransom, R.A. Michael, V.W. Macdonald. A randomized controlled trial evaluating recovery and survival of 6% dimethyl sulfoxide-frozen autologous platelets in healthy volunteers, *Transfusion* 2013; 53:128-137.

[91] L.N. Johnson, K.M. Winter, S. Reid, T. Hartkopf-Theis, D.C. Marks. Cryopreservation of buffy-coat-derived platelet concentrates in dimethyl sulfoxide and platelet additive solution, *Cryobiology* 2011; 62:100-106.

[92] S.F. Khuri, N. Healey, H. MacGregor, M.R. Barnard, I.O. Szymanski, V. Birjiniuk, A.D. Michelson, D.R. Gagnon, C.R. Valeri. 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; 117:172-183; discussion 183-174.

[93] C.C. Lelkens, J.G. Koning, B. de Kort, I.B. Floot, F. Noorman. Experiences with frozen blood products in the Netherlands military, *Transfus Apher Sci* 2006; 34:289-298.

[94] M.C. Reade, D.C. Marks, L. Johnson, D.O. Irving, A.D. Holley. Frozen platelets for rural Australia: the CLIP trial, *Anaesth Intensive Care* 2013; 41:804-805.

[95] H.F. Pidcoke, P.C. Spinella, A.K. Ramasubramanian, G. Strandenes, T. Hervig, P.M. Ness, A.P. Cap. Refrigerated platelets for the treatment of acute bleeding: a

review of the literature and reexamination of current standards, *Shock* 2014; 41 Suppl 1:51-53.

[96] P.M. Nair, H.F. Pidcoke, A.P. Cap, A.K. Ramasubramanian. Effect of cold storage on shear-induced platelet aggregation and clot strength, *J Trauma Acute Care Surg* 2014; 77:S88-93.

[97] C.S. Manno, K.W. Hedberg, H.C. Kim, G.R. Bunin, S. Nicolson, D. Jobes, E. Schwartz, W.I. Norwood. Comparison of the hemostatic effects of fresh whole blood, stored whole blood, and components after open heart surgery in children, *Blood* 1991; 77:930-936.

[98] G.A. Becker, M. Tuccelli, T. Kunicki, M.K. Chalos, R.H. Aster. Studies of platelet concentrates stored at 22 C nad 4 C, *Transfusion* 1973; 13:61-68.

[99] H.H. Wandall, K.M. Hoffmeister, A.L. Sorensen, V. Rumjantseva, H. Clausen, J.H. Hartwig, S.J. Slichter. Galactosylation does not prevent the rapid clearance of long-term, 4 degrees C-stored platelets, *Blood* 2008; 111:3249-3256.

[100] J.S. Jhang, S.L. Spitalnik. Glycosylation and cold platelet storage, *Curr Hematol Rep* 2005; 4:483-487.

[101] M.G. Egidi, A. D'Alessandro, G. Mandarello, L. Zolla. Troubleshooting in platelet storage temperature and new perspectives through proteomics, *Blood Transfus* 2010; 8 Suppl 3:s73-81.

[102] K.M. Hoffmeister, E.C. Josefsson, N.A. Isaac, H. Clausen, J.H. Hartwig, T.P. Stossel. Glycosylation restores survival of chilled blood platelets, *Science* 2003; 301:1531-1534.

[103] J. Cancelas. Exploring new avenues for cold storage of platelets. *The 27th Regional Congress of the International Society of Blood Transfusion*. Copenhagen, Denmark: International Society of Blood Transfusion; 2017.

[104] B.A. Badlou, Y.P. Wu, W.M. Smid, J.W. Akkerman. Platelet binding and phagocytosis by macrophages, *Transfusion* 2006; 46:1432-1443.

[105] F. Ferrer, J. Rivera, M.L. Lozano, J. Corral, V.V. Garcia. Effect of cold-storage in the accumulation of bioreactive substances in platelet concentrates treated with second messenger effects, *Haematologica* 2001; 86:530-536.

[106] D.H. Lee, M.A. Blajchman. CHAPTER 70 - Platelet Substitutes and Novel Methods of Platelet Preservation A2 - Michelson, Alan D. *Platelets (Second Edition)*. Burlington: Academic Press; 2007, p. 1297-1309.

[107] J. Connor, L.M. Currie, H. Allan, S.A. Livesey. Recovery of in vitro functional activity of platelet concentrates stored at 4 ° C and treated with second-messenger effectors, *Transfusion* 1996; 36:691-698.

[108] K. Reddoch Cardenas, C. Cantu-Garza, H.F. Pidcoke, A.P. Cap. Specialized Proresolving Mediators (SPMs) Help to Alleviate the Cold Platelet Storage Lesion, *Blood* 2017; 130:1121-1121.

[109] N.S. Barteneva, E. Fasler-Kan, M. Bernimoulin, J.N. Stern, E.D. Ponomarev, L. Duckett, I.A. Vorobjev. Circulating microparticles: square the circle, *BMC Cell Biol.* 2013; 14:23.

[110] M. Weiss, J.P. Frohnmayer, L.T. Benk, B. Haller, J.W. Janiesch, T. Heitkamp, M. Borsch, R.B. Lira, R. Dimova, R. Lipowsky, E. Bodenschatz, J.C. Baret, T. Vidakovic-Koch, K. Sundmacher, I. Platzman, J.P. Spatz. Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics, *Nat Mater* 2017.

[111] M.G. Van der Planken, F. Vertessen, E. Mortelmans, D. Van Bockstaele, L. Muylle, G. Mertens, Z.N. Berneman. The evolution of platelet procoagulant activity of remnant platelets in stored platelet concentrates prepared by the platelet-rich plasma method and the buffy coat method, *Ann Hematol* 1999; 78:1-7.

[112] P. Metcalfe, L.M. Williamson, C.P. Reutelingsperger, I. Swann, W.H. Ouwehand, A.H. Goodall. Activation during preparation of therapeutic platelets affects deterioration during storage: a comparative flow cytometric study of different production methods, *Br J Haematol* 1997; 98:86-95.

[113] G. Paglia, O.E. Sigurjonsson, O. Rolfsson, M.B. Hansen, S. Brynjolfsson, S. Gudmundsson, B.O. Palsson. Metabolomic analysis of platelets during storage: a comparison between apheresis- and buffy coat-derived platelet concentrates, *Transfusion* 2015; 55:301-313.

[114] T. Keegan, A. Heaton, S. Holme, M. Owens, E. Nelson, R. Carmen. Paired comparison of platelet concentrates prepared from platelet-rich plasma and buffy coats using a new technique with 111In and 51Cr, *Transfusion* 1992; 32:113-120.

Table 1: Platelet granule contents and function in haemostasis

Granule	Class	Components	Function
α	Large adhesive proteins	vWF Thrombospondin-1 Vitronectin Fibronectin	Platelet aggregation
	Mitogenic factors	VEGF TGF-β PDGF	Wound repair
	Coagulation factors	Factors V, VII, XII, XII	Coagulation
	Protease inhibitors	Protein C PAI-1 TFPI	Anticoagulation
	Soluble immune factors	PF4 RANTES β-thromboglobulin/NAP-2 sCD40L	Inflammation
Dense	Dense granule contents	ADP, ATP Serotonin Calcium pyrophosphate	Platelet activation/aggregation Platelet activation/aggregation Coagulation

Abbreviations: ADP=adenosine diphosphate, ATP=adenosine triphosphate, NAP-2=neutrophil activating peptide-2, PAI-1=plasminogen activator inhibitor, PDGF=platelet derived growth factor, PF4=platelet factor 4, RANTES=regulated on activation, normal T cell expressed and secreted, sCD40L=soluble CD40 ligand, TFPI=tissue factor pathway inhibitor, TGF-β=transforming growth factor β, VEGF=vascular endothelial growth factor, vWF=von Willebrand factor

Table 2: Indications for platelet transfusion

Prophylactic	Treatment

• Hypoproliferative thrombocytopenia
• Increased risk of bleeding (platelets <5,000/μl)
• Bone marrow failure
  ○ Platelets <10,000/μl if no risk factors
  ○ Platelets <20,000/μl if fever, on antibiotics, evidence of systemic haemostatic failure
• Surgery/invasive procedure
  ○ Aim to keep platelets >50,000/μl
• High risk of bleeding
  ○ Aim to keep platelets >100,000/μl
	• Bleeding where thrombocytopenia is major contributing factor
	• Massive haemorrhage/transfusion
	  ○ As part of massive transfusion protocol

Table 3: Differences in PSL by PC preparation method

PSL	AP PCs	BC PCs	PRP PCs
MPV[19, 111]	↓ over 5 days	No change	No change (PRP PC< BC PC)
PRW[19]	↓ over 5 days	No change	-
% depolarized MMP[19]	No change	↑ over 5 days	-
GPIbα[19, 112]	↓ over 7 days (AP PC <BC PC)	↓ over 7 days	↓ over 7 days (PRP PC < AP PC)
CD47[19]	No change (AP PC >BC-PC)	No change	-
GPIIIa[19]	No change (AP PC >BC-PC)	No change	-
Platelet activation[112, 113]	↑ over 5 days (AP PC >BC-PC)	↑ over 5 days	↑ over 5 days (PRP PC >AP-PC)
Metabolism[113, 114]	↑ Glucose consumption + ↑ lactate secretion (AP PC > BC PC = PRP PC)		
Abbreviations: AP PC=apheresis platelet concentrates; BC PC=buffy coat platelet concentrates; MMP=mitochondrial membrane potential; MPV=mean platelet volume; PRP PC = platelet rich plasma platelet concentrates, PRW=platelet redistribution width			

Figure 1 – Role of platelets in thrombus formation. Platelets activate on contact with subendothelial proteins (collagen, von Willebrand's factor) to express P-selectin and release microparticles, α granules and dense granules. Activated platelets aggregate in response to collagen, epinephrine, ADP, thrombin, fibrinogen, von Willebrand's factor, platelet activating factor and thrombin receptor-activating protein-6. Microparticles, tissue factor and activated factor V released by platelets enhance thrombin generation to provide positive feedback for thrombus generation.

Figure 2 – Platelet concentrate preparation methods. Platelet rich plasma (PRP) PCs utilises soft centrifugation to first separate platelets and plasma from red and white blood cells. Hard centrifugation subsequently pellets platelets. In contrast, buffy coat (BC) PCs are produced via an initial hard centrifugation to concentrate platelets into the BC, which undergoes soft centrifugation to remove remaining red blood cells. Platelets from four to six donors are pooled to produce a therapeutic dose of PRP or BC PCs ($>3x10^{11}$ platelets). For apheresis PCs, whole blood is collected into an automated cell separator which centrifuges whole blood and collects platelets, while returning other components to the donor. As 3.5L of donor blood can be processed per collection, each apheresis PC unit is sourced from a single donor. Platelets from BC and apheresis PCs are resuspended in a mixture of platelet additive solution and plasma, whereas, PRP BCs remain suspended in plasma only.

Figure 3 – Room-temperature (RT) PSLs and management strategies (blue). ADP=adenosine diphosphate, DINCH-PVC=1,2-cyclohexane-dicarboxylic acid diisonoylester-polyvinyl chloride, HSR=hypotonic shock response, LDH=lactic dehydrogenase, NO=nitric oxide, NAAGA=*N*-acetylaspartylglutamic acid, PAR=protease-activated receptors, PC=platelet concentrate, PMP=platelet microparticles, TCA=tricarboxylic acid

Figure 4 – Platelet metabolism and associated PSLs. Glucose enters from the surrounding environment and can undergo glycolysis (non-oxidative) in the cytoplasm to produce pyruvate and a small amount of ATP. Pyruvate participates in oxidative phosphorylation in the mitochondrial matrix via the tricarboxylic acid cycle. The citric acid cycle produces electron donors (NADH + $FADH_2$) which are fed into the electron transport chain for bulk ATP production. Proteins at the mitochondrial membrane pump hydrogen into the intermembrane space to generate the mitochondrial membrane potential. The mitochondrial

membrane potential drives the ATP synthase to produce ATP which is required for platelet function. During PC storage, glycolysis is upregulated while mitochondrial function is suppressed. This leads to increased glucose consumption and lactic acid secretion along with, decreased ATP production. Ultimately, these changes are linked to storage media acidification, decreased platelet survival and aggregation.

Figure 5 – Immune system effects of PSLs. Platelets and contaminating leukocytes in stored platelet concentrates (PCs) mediate immune system dysfunction via cytokines and platelet microparticles (PMPs). These constituents combine to cause non-haemolytic transfusion reactions, transfusion-related lung injury and transfusion-related immunomodulation. The latter two reactions require a "1st hit" such as endotoxaemia which has been simulated by lipopolysaccharide (LPS) addition *in vitro*.

Figure 6 – Cold storage PSLs and management strategies (blue). Ca^{2+}=calcium.

Highlights

- Platelet storage lesions (PSLs) form during platelet concentrate preparation and storage
- PSLs include storage media changes (such as nutrient depletion and waste accumulation), platelet marker alterations and microparticle formation
- PSLs affect platelet activation, aggregation and immune function
- PSLs lead to decreased transfusion efficacy but do not affect transfusion safety
- Future research involve vascular flow models, storage media and temperature optimisation

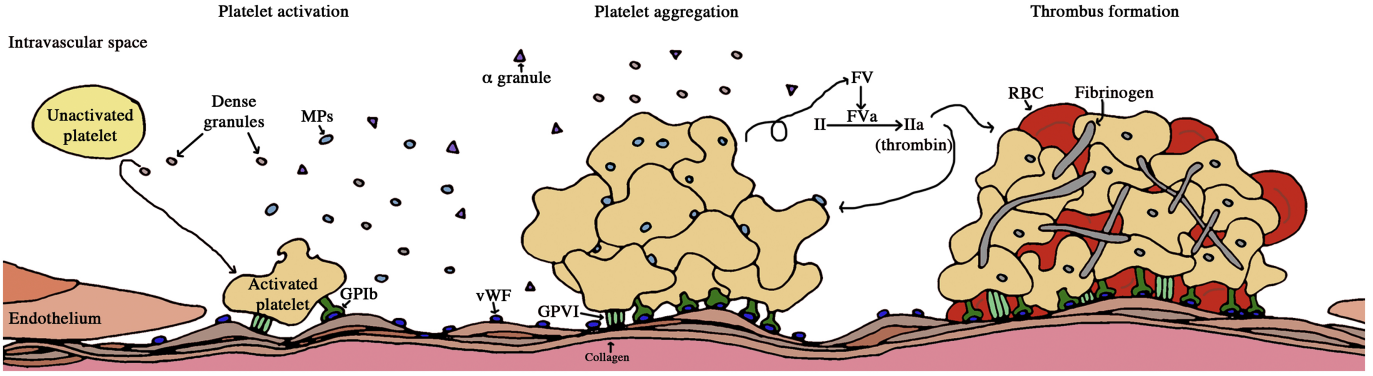
Platelet activation
Platelet aggregation
Thrombus formation
Intravascular space
Unactivated platelet
Dense granules
MPs
α granule
FV
FVa
II
IIa
(thrombin)
RBC
Fibrinogen
Activated platelet
GPIb
vWF
GPVI
Collagen
Endothelium

Figure 1

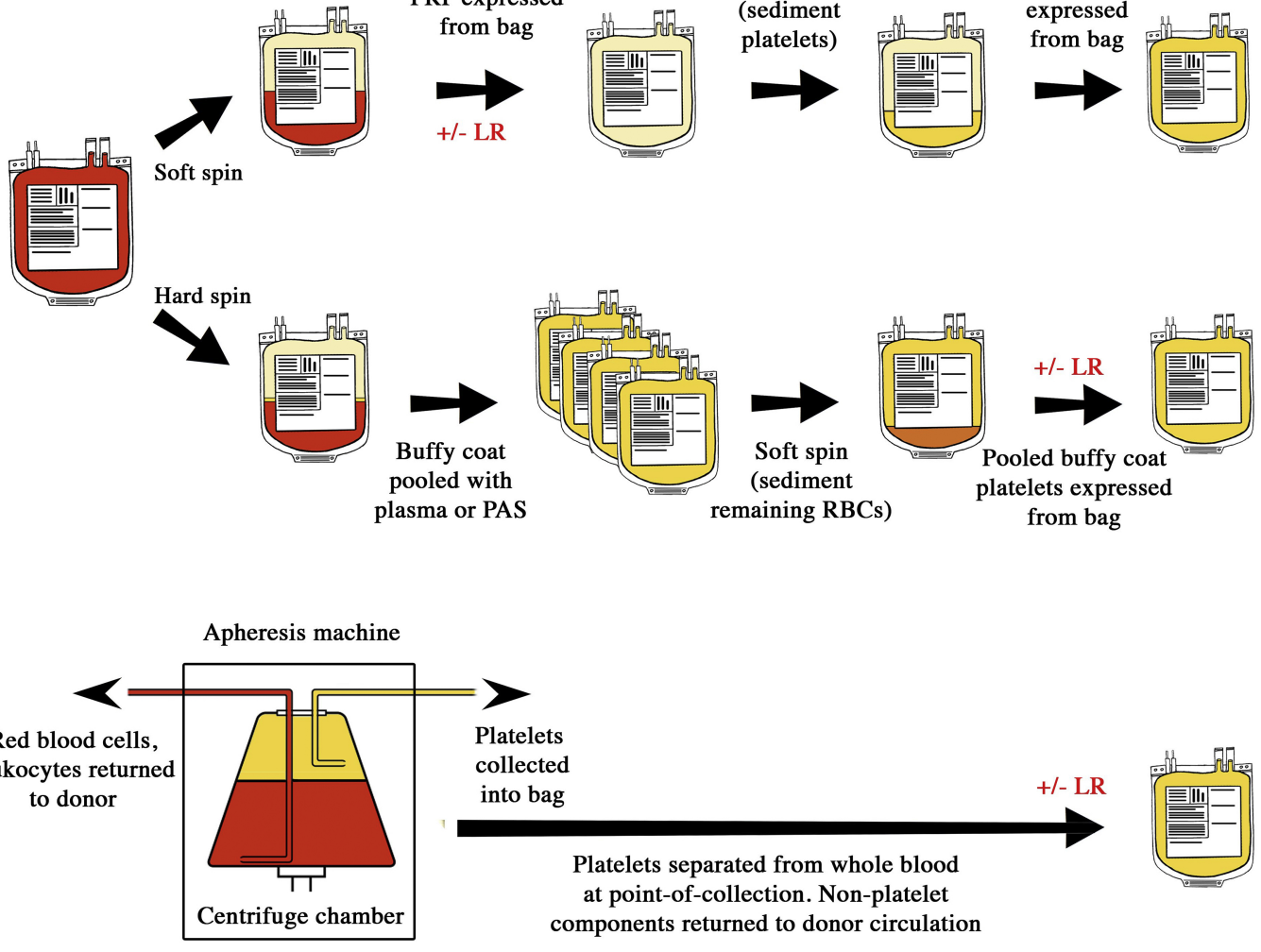
PRP expressed from bag
+/- LR
Hard spin (sediment platelets)
Plasma expressed from bag
Soft spin
Hard spin
Buffy coat pooled with plasma or PAS
Soft spin (sediment remaining RBCs)
+/- LR
Pooled buffy coat platelets expressed from bag
Apheresis machine
Red blood cells, leukocytes returned to donor
Platelets collected into bag
Centrifuge chamber
+/- LR
Platelets separated from whole blood at point-of-collection. Non-platelet components returned to donor circulation

Figure 2

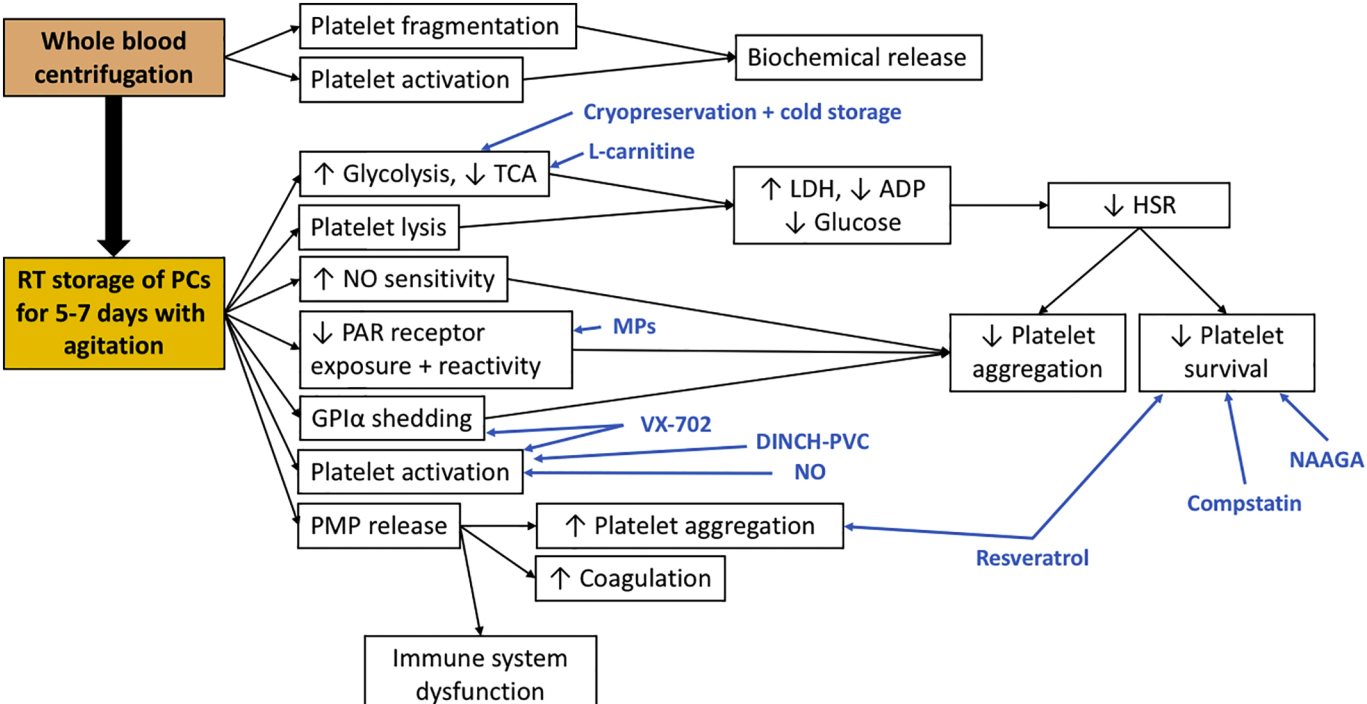
Whole blood centrifugation
Platelet fragmentation
Platelet activation
Biochemical release
Cryopreservation + cold storage
L-carnitine
RT storage of PCs for 5-7 days with agitation
↑ Glycolysis, ↓ TCA
Platelet lysis
↑ LDH, ↓ ADP
↓ Glucose
↓ HSR
↑ NO sensitivity
↓ PAR receptor exposure + reactivity
MPs
↓ Platelet aggregation
↓ Platelet survival
GPIα shedding
VX-702
DINCH-PVC
NO
Platelet activation
NAAGA
Compstatin
PMP release
↑ Platelet aggregation
↑ Coagulation
Resveratrol
Immune system dysfunction

Figure 3

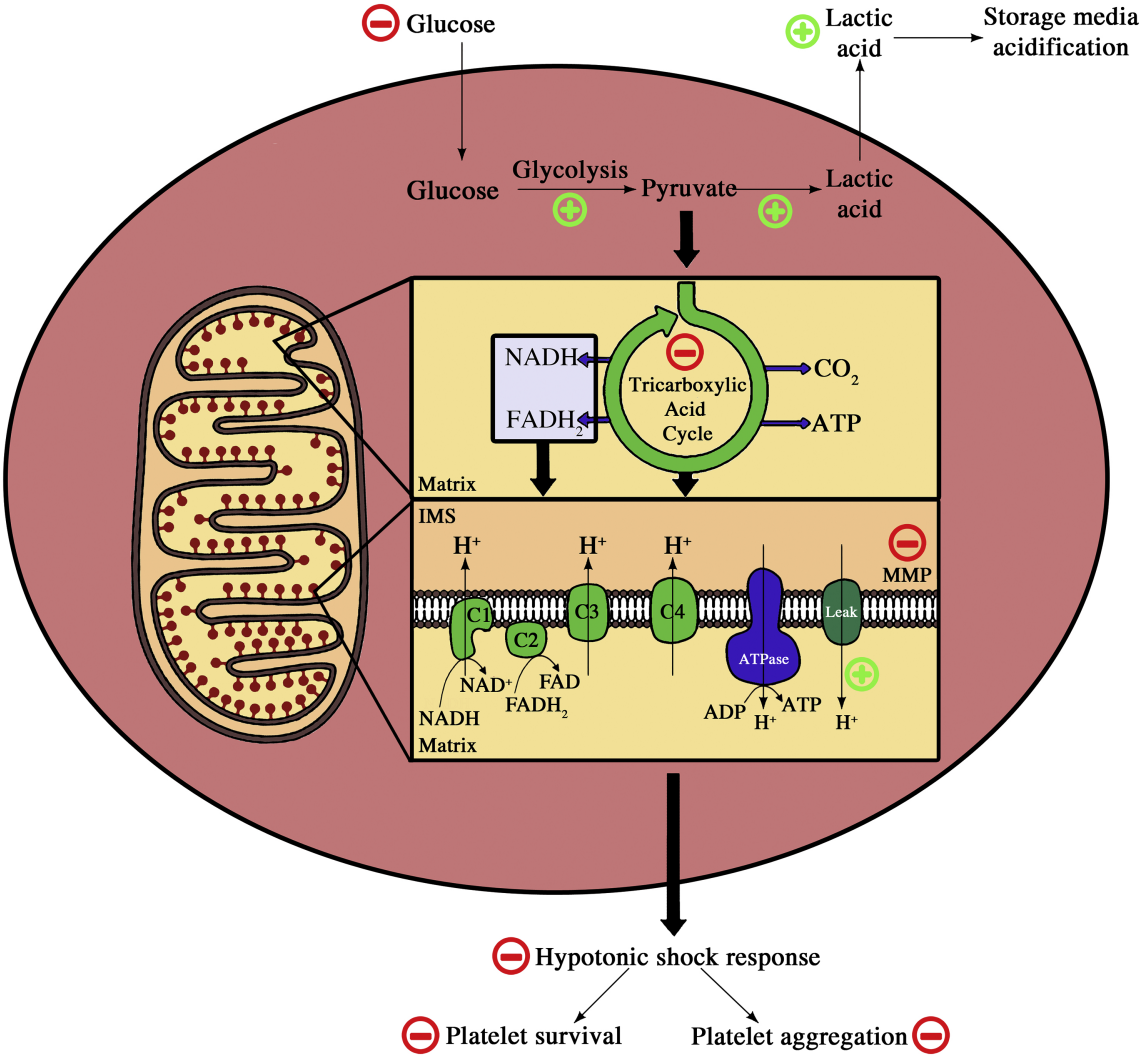
Glucose
Lactic acid
Storage media acidification
Glucose
Glycolysis
Pyruvate
Lactic acid
NADH
FADH2
Tricarboxylic Acid Cycle
CO2
ATP
Matrix
IMS
H+
H+
H+
MMP
C1
C2
C3
C4
Leak
ATPase
NAD+
FAD
FADH2
NADH
Matrix
ADP
H+
ATP
H+
Hypotonic shock response
Platelet survival
Platelet aggregation

Figure 4

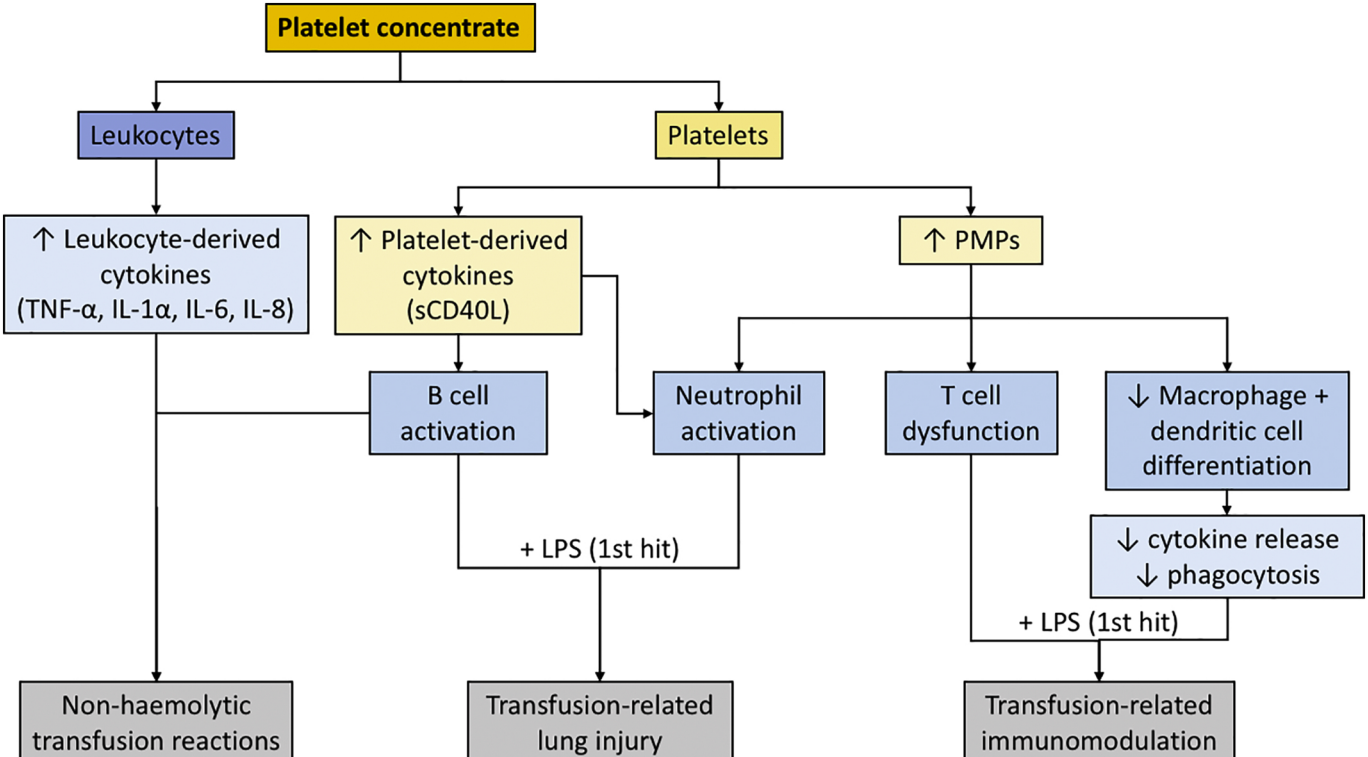
Platelet concentrate
Leukocytes
Platelets
↑ Leukocyte-derived cytokines (TNF-α, IL-1α, IL-6, IL-8)
↑ Platelet-derived cytokines (sCD40L)
↑ PMPs
B cell activation
Neutrophil activation
T cell dysfunction
↓ Macrophage + dendritic cell differentiation
↓ cytokine release
↓ phagocytosis
+ LPS (1st hit)
+ LPS (1st hit)
Non-haemolytic transfusion reactions
Transfusion-related lung injury
Transfusion-related immunomodulation

Figure 5

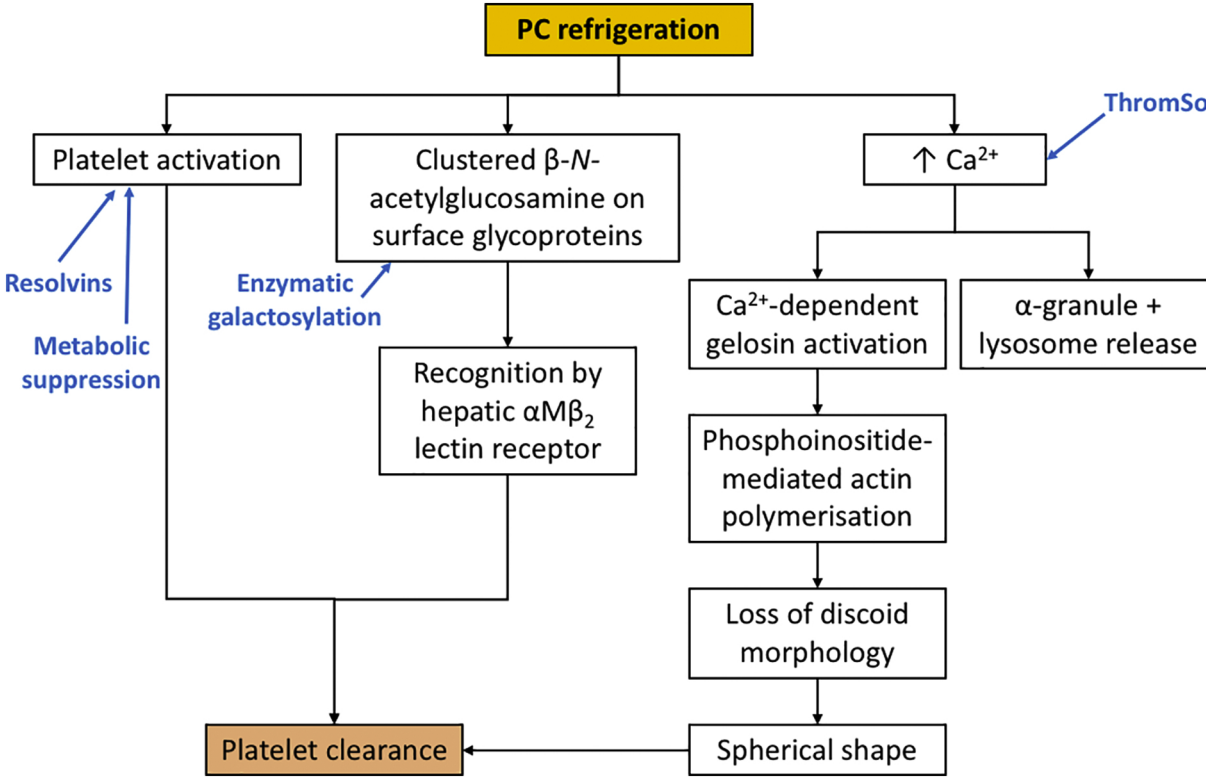
PC refrigeration
Platelet activation
Clustered β-*N*-acetylglucosamine on surface glycoproteins
↑ Ca^{2+}
ThromSol
Resolvins
Metabolic suppression
Enzymatic galactosylation
Recognition by hepatic $\alpha M\beta_2$ lectin receptor
Ca^{2+}-dependent gelosin activation
α-granule + lysosome release
Phosphoinositide-mediated actin polymerisation
Loss of discoid morphology
Spherical shape
Platelet clearance

Figure 6