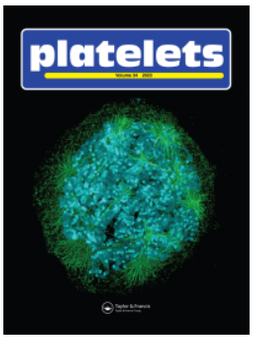

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REVIEW

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Cold stored platelets in the management of bleeding: is it about bioenergetics?

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Abstract

When platelet concentrates (PCs) were first introduced in the 1960s as a blood component therapy, they were stored in the cold. As platelet transfusion became more important for the treatment of chemotherapy-induced thrombocytopenia, research into ways to increase supply intensified. During the late 1960s/early 1970s, it was demonstrated through radioactive labeling of platelets that room temperature platelets (RTP) had superior post-transfusion recovery and survival compared with cold-stored platelets (CSP). This led to a universal switch to room temperature storage, despite CSP demonstrating superior hemostatic effectiveness upon being transfused. There has been a global resurgence in studies into CSP over the last two decades, with an increase in the use of PC to treat acute bleeding within hospital and pre-hospital care. CSP demonstrate many benefits over RTP, including longer shelf life, decreased bacterial risk and easier logistics for transport, making PC accessible in areas where they have not previously been, such as the battlefield. In addition, CSP are reported to have greater hemostatic function than RTP and are thus potentially better for the treatment of bleeding. This review describes the history of CSP, the functional and metabolic assays used to assess the platelet storage lesion in PC and the current research, benefits and limitations of CSP. We also discuss whether the application of new technology for studying mitochondrial and glycolytic function in PC could provide enhanced understanding of platelet metabolism during storage and thus contribute to the continued improvements in the manufacturing and storage of PC.

Plain Language Summary

What is the context?

- To transition into an activated state, platelets require a highly efficient source of energy that is met through the production of ATP – this is referred to as "platelet bioenergetics"
- Platelets can be removed from healthy donors and used to make platelet concentrates for clinical use
- Platelet concentrates are used clinically either therapeutically (to halt bleeding) or prophylactically (to prevent bleeding in patients with low platelet counts)
- They are stored at room temperature (20–24°C) with constant gentle agitation, in packs that allow gas exchange and have a 7-day shelf life in some jurisdictions
- Storing platelets in the cold (2–6°C) has historically been shown to improve their ability to halt bleeding

What is new?

- There is a renewed interest in cold stored platelets for use in actively bleeding patients
- There are benefits to cold-storing platelets over room temperature storage
- Cold stored platelets are licensed in the US and Norway for certain indications for 14 days

What is next?

- Cold stored platelets have the potential to improve logistics of clinical supply of platelets, enable supply of platelet concentrates where access is currently limited, such as pre-

Keywords

Cold storage, platelet bioenergetics, platelet concentrate, platelet storage lesion, platelet transfusion, room-temperature stored platelets

History

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hospital care and on the battlefield and provide improved hemostatic effects for bleeding patients.
- New research measuring the bioenergetic profiles of cold stored platelets could advance understanding of metabolism in cold stored platelets and support decisions on their re-introduction on a wider scale

Introduction

Platelets are small, anucleate cells that are released from megakaryocytes in the bone marrow and lungs. They are the smallest of the blood cells, with the normal peripheral platelet count being in the range of 150 to 400×10^9/L [1]. In the absence of a nucleus, it is likely that the platelet circulation lifespan of 7–10 days [2] is largely determined by the health of their mitochondria [3]. Platelets circulate in a quiescent state toward the edges of blood vessels (margination) where they are ideally placed to respond rapidly to vessel damage [4]. Following damage to the vessel wall, platelets are captured from the circulation and adhere to the extracellular matrix, become activated, release their granule contents and aggregate, resulting in the formation of a platelet plug [5].

The biomechanical transition from a quiescent state to an activated state requires a highly efficient source of energy. This bioenergetic demand is met through the production (and subsequent hydrolysis) of adenosine triphosphate (ATP), using a metabolic system that combines glycolysis and oxidative phosphorylation (OXPHOS) [3,6].

Glycolysis is independent of oxygen and takes place in the cytoplasm of the cell where ATP is produced by glucose being split into pyruvate. The pyruvate is either broken down by lactate dehydrogenase into lactate or is transported across the mitochondrial membrane and converted into acetyl coenzyme A (Acetyl-CoA). Acetyl-CoA is the starting material for the citric acid cycle which harvests energy in the form of the reduced compounds NADH and $FADH_2$. The NADH & $FADH_2$ deposit their electrons into the electron transport chain (ETC) (Figure 1) to generate ATP through the oxygen-dependent process of OXPHOS [7].

The production and consumption of energetic substrates in platelets is referred to as "platelet bioenergetics" [8]. Platelet bioenergetics is not a simple dichotomy between the processes of glycolysis and OPHOS, as platelets demonstrate considerable metabolic plasticity between the two avenues [8]. At a basal state, both OXPHOS and glycolysis play a role in energy production in platelets [9]. However, there appears to be some discrepancy in the literature around the amount of ATP supplied by glycolysis and the amount supplied by OXPHOS in a resting state, with Wang and colleagues suggesting approximately 60% from glycolysis & 40% from OXPHOS [10], Kilkson and colleagues suggesting a 15%:85% ratio [11] and Reddoch-Cardenas suggesting a 25%:75% ratio [12].

The degree to which platelets use glycolysis or OXPHOS is likely a balance between the need to generate ATP quickly, favoring the high rate but low yield state of glycolysis, as opposed to the low rate but higher ATP yield of OXPHOS [13]. The transition of platelets to an activated state promotes rapid uptake of exogenous glucose, associated with a shift to energy generation predominately through glycolysis with a minor rise in mitochondrial oxygen consumption [6]. Platelet adhesion is one of the few energy-independent biomechanical processes [14], whereas platelet spreading and aggregation have been shown to be primarily fueled by glycolysis, while platelet contraction shows more of an association with OXPHOS [8]. This metabolic flexibility allows platelets to utilize glycolysis instead of OXPHOS so as to adapt to different situations, such as localized hypoxia [3].

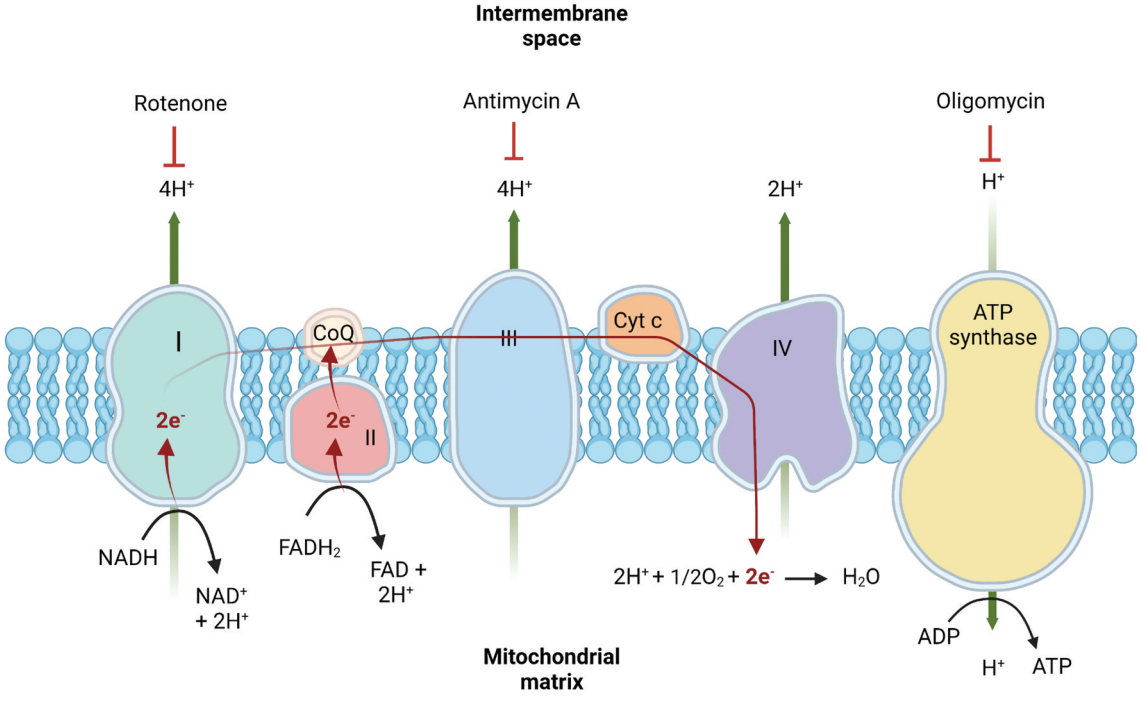

Figure 1. Basic schematic of the electron transport chain (ETC). Key parameters of mitochondrial function can be measured through the use of different modulators of respiration (oligomycin, rotenone and antimycin) to isolate different parts of the ETC. Figure created with Biorender.com.

Clinical use of platelet concentrates

Platelet component therapy (platelet transfusions) as an alternative to whole blood transfusion was first introduced in the 1960s and revolutionized the treatment of thrombocytopenic patients [15]. Platelet transfusions are generally categorized into prophylactic treatment (to prevent bleeding in thrombocytopenic patients) or therapeutic treatment (to treat active bleeding). Prophylactic platelet transfusions are predominantly used in chemotherapy patients who require the maximum platelet circulation time to increase intervals between transfusions and reduce the risk of alloimmunization. Conversely, platelet transfusion to treat bleeding and regain hemostatic control, for example in trauma patients, requires fast initiation of the clot, which requires platelets that are able to activate and function rapidly after transfusion [16].

Approximately 67% of platelet transfusions are used for prophylactic management of hematological disorders/conditions with the remainder being used therapeutically [17]. However, there is a shift away from prophylactic platelet transfusion with the introduction of lower transfusion triggers (10×10^9/L instead of 20×10^9/L) being implemented in acute leukemia's [18]. In addition, there have been several studies indicating that patients who have undergone autologous stem cell transplants and are haemostatically stable can be managed safely without prophylactic platelet transfusion [19,20] reducing use of platelet concentrates (PC) in this cohort. In addition, evidence-based guidelines on platelet transfusion are advocating restrictive use of prophylactic platelet transfusions to patients by maintaining lower platelet count triggers for transfusion and employing a one PC dose for routine prophylactic transfusion strategy [17].

As well as clinical changes in appropriate use for prophylaxis, there has been increasing use of PC to treat acute bleeding in traumatic hemorrhage and surgery as well as other causes of bleeding [21]. Within the UK, there has been an intense focus on improving trauma care by setting up regional trauma networks which contain major trauma centers [22]. This has led to a more consistent and organized approach to trauma care. The foundation of damage control resuscitation for trauma patients with major hemorrhage is that early correction of coagulopathy limits blood loss and decreases blood product transfusion [23].

The UK BSH guideline for the hematological management of major hemorrhage [22] recommends that initially in trauma, there should be empirical use of red cells and plasma, usually given in a ratio of 1:1 and that platelets should be transfused to maintain the platelet count at $>50\times10^9$/L or at higher threshold in patients with intracranial bleeding/spinal bleeding or in actively bleeding patients with falling platelet counts [22]. A sub-study of the Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial [24] has since demonstrated that early platelet administration in severely injured trauma patients is independently associated with improved hemostasis and reduced mortality, suggesting that a ratio of 1:1:1 should be used in all trauma cases with major bleeding. Taking these factors into consideration, there is an expectation that there will be a reduction in prophylactic transfusions and an increase in therapeutic platelet transfusions going forward.

Manufacture and storage of platelet concentrates

There are two main types of PC used for transfusion: buffy coat derived pooled PC and apheresis derived PC with usage of the two components varying greatly between countries. Pooled platelets are generated from whole blood donations which are centrifuged to separate the red cells, buffy coat (containing platelets and white cells) and the plasma. In the UK, four buffy coats are pooled together with a platelet additive solution (PAS) to generate a PC suspended in approximately 35%:65% plasma:PAS.

Apheresis PC are collected using licensed plateletpheresis platforms which remove venous whole blood from the arm of a volunteer donor, automatically separating the platelets from the other blood cells and plasma through centrifugation. The platelets are diverted into a collection pack, along with a portion of plasma, whilst the remainder of the blood is returned to the donor.

Pooled and apheresis PC are prepared into specially designed platelet storage packs that allow gaseous exchange with the environment and can generally be used interchangeably for transfusion purposes, with no evidence of difference in clinical efficacy between the two for room temperature storage [15]. The exceptions to this in the UK are for neonatal transfusion, where apheresis PC are used to reduce donor exposure, and thus the risk of a transfusion-transmitted infection, and when donor and recipient need to be matched for HLA antigens.

Platelets are logistically more challenging to store than other blood components as they require continuous gentle agitation at 22°C ± 2°C (room temperature (RT)) in specialized incubators and agitators [25] to prevent platelet aggregation and maintain aerobic metabolism [26]. The temperature and method of storage provides ideal conditions for bacterial proliferation with bacterial contamination of PC ranging from 1:1000 to 1:2500 [27].

The risk of bacterial contamination is a major factor in the shelf life of PC being limited. In some countries, such as the UK, these risks are mitigated by bacterial culturing of all PC, allowing their shelf life to be extended from five to seven days. However, bacterial culturing systems have their shortcomings, and bacterial contamination can be missed [28]. The short shelf life, even when extended to seven days makes stock management of PC challenging and can result in considerable component wastage through time expiry.

The platelet storage lesion

In addition to the risk of bacterial growth, another reason for the short shelf life of PC is that they begin to show evidence of a significant loss of platelet function *in vitro* during storage, which may affect the efficacy of the platelets once transfused into patients [29]. This decline in function throughout storage is referred to as the "platelet storage lesion" (PSL).

The PSL is a complex biological event, involving a series of biochemical, structural and functional changes that occur to platelets as result of blood collection, mechanical manipulation during manufacture and storage conditions [30,31]. It is important to understand these changes as they are associated with reduced *in vivo* platelet survival [32], and if they could be reduced, the shelf life and availability of PC may be improved.

The manufacture of PC exposes them to stresses, such as centrifugation, manipulation, suspension in chemical storage medium and exposure to foreign surfaces such as the plastic of blood bags [33], as well as the loss of protection that is conferred by the endothelium when in the circulation. All of these stressors can cause physiological responses that resemble platelet activation [29,30], hence many of the assays used to study the PSL are functional assays similar to those used to study platelet activation for diagnostic purposes in patients. PC are currently stored in a medium that enables them to be metabolically active, at 20–24°C with constant agitation, in a pack that enables gaseous exchange. During storage at this temperature, glycolysis is enhanced and mitochondrial function is reduced leading to glucose depletion, increased lactate production and a resulting acidification of the PC [30]. For this reason, assessment of platelets during storage often includes and benefits from measures of metabolism and mitochondrial health.

Table I. Commonly used assays to measure the PSL in RTP.

Type	Assay	Purpose	Effect of PSL on RTP
Morphological	Visual inspection of swirling	Measures disk to sphere shape change in platelets. Discoid platelets when rocked gently against a light source scatter light in different directions, causing the phenomenon known as 'swirling.'	Lack of swirling [29,35]
	Platelet morphology score	Visual assessment of platelet morphology using phase-contrast microscopy. Quantified using scoring system such as Kunicki Morphology Score	Platelets lose their discoid shape [12,36,37]
Functional	Platelet aggregation in response to agonists	Measures platelets responsiveness to different agonists, e.g. thrombin, collagen, epinephrine and ADP	Ability to respond to agonists declines [38,39]
	Hypotonic Shock Response	Measures the ability of the platelet to return to its normal shape after hypotonic challenge.	Declines [40,41]
	Extent of Shape Change	Measures the amount of shape change that the platelet undergoes in response to a pre-set dose of ADP	Declines [29,42]
	CD62P/P-selectin surface expression	Monitors platelet degranulation. Flow cytometric assay for platelet activation markers released from alpha granules and subsequently expressed on the surface of platelet	Enhanced exposure [43,44]
	Annexin V binding	Flow cytometric assay using Annexin V to monitor exposure of anionic phospholipids, such as phosphatidyl serine on the platelet membrane	Enhanced exposure (also an indicator of apoptosis) [44,45]
	Soluble CD62P	Measures levels of CD62P shed from the platelet membrane by an ELISA technique	Increases during storage [29,30]
	Thrombin generation	Measures kinetics of thrombin generation in response to tissue factor stimulus. Measured by a calibrated automated thrombogram	Thrombin generation indicators suggest platelets become more procoagulant [46]
Metabolic	Lactate	Measures metabolism of platelets. Lactate is generated by glycolysis	Increases during storage while glucose is available [30,43,47]
	Glucose	Measures metabolism of platelets. Glucose is broken down to pyruvate and lactate by glycolysis	Depletes during storage [43,47]
	pH	pH meter – measures level of acidity in PC	Decreases (increased acidification secondary to glycolysis) [29]
	pO_2 and pCO_2	Measured to ensure that sufficient gas exchange is occurring during storage	While platelets are metabolically active, O_2 declines, CO_2 increases [48]
	Mitochondrial Membrane Potential	Flow cytometric assay. Key indicator of cell health – results are relatable to cells capacity to generate ATP by oxidative phosphorylation	Depolarises and thus decreases as mitochondrial function is impaired [49]
	Extracellular ATP	Measures ATP-dependent oxidation of luciferin	Decreases during storage, suggestive of a deficiency in glycolysis &/or OXPHOS [50]

The PSL has been the focus of intense research. Despite earlier hopes of finding a simple assay that could reliably predict the function and survival of stored platelets post-transfusion, the complex nature of the PSL means there is no single assay which can accurately predict the efficacy of a platelet transfusion [34].

Table I outlines the commonly used assays to study the PSL and the effect that the PSL has on the assay results for room temperature stored platelets (RTP).

The PSL is characterized by platelet activation during storage: α-granule contents are released into the media (soluble CD62P), expression of CD62P and phosphatidylserine on the platelet membrane is increased and platelet shape change occurs, including an increase in the Mean Platelet Volume (MPV) and a lack of the "swirling" phenomenon [51].

The ability of PC to aggregate is assessed using platelet aggregometry in response to agonists. Platelet aggregometry has been reported to correlate well with clinically relevant outcomes like bleeding and thromboembolic events in clinical settings [52]. During the PSL, the ability of platelets to respond to agonists and to aggregate significantly decreases, suggesting a likely decrease in *in vivo* function post-transfusion, although there is evidence to suggest that decreased function may be reversed in the days following transfusion [53].

As PC are metabolically active due to their storage temperature and gas-permeable bag, many studies of the PSL examine measures of metabolic function. During storage, PC utilize glucose and accumulate lactate as a result of glycolysis. As such, measuring lactate and glucose can give an indication of the amount of glycolysis occurring in the cells, but it is not a direct measure. Lactate generation results in hydrogen ions being produced which acidifies the PC (reducing the pH) causing the platelets to swell and lose their discoid shape. Once the pH falls below 6.1, the return to the original shape is not possible [54].

Metabolomics analysis [47] has demonstrated that glucose is exclusively converted to lactate via glycolysis with decreased mitochondrial function during the first three days of storage but that toward the end of shelf life, OXPHOS increases to generate ATP when substrates for glycolysis become depleted [30,47]. It is plausible that if metabolism (and the active glycolytic pathway) can be reduced during storage of PC, that the effects of the PSL could also be reduced, leading to a better quality product with a prolonged shelf life. One potential way of reducing metabolism in PC is to store them at refrigerated temperatures.

Cold stored platelets

Prior to 1969, PC were stored at 4°C with a very limited shelf life due to the view that they should be transfused within a few hours of being isolated from the donor [55]. However, as PC became increasingly important as a therapy for the treatment of malignant disorders requiring intensive chemotherapy, pressure increased to

produce them in adequate quantities to meet demand [55]. This in turn drove research into alternative methods of storage which could prolong shelf life and improve supply.

A watershed came in the late 1960s/1970s with the publication of several critical studies comparing cold-stored platelets (CSP) with room-temperature stored platelets (RTP) [55–57]. Microdoses of CSP and RTP were labeled with radioactive chromium (^{15}Cr), transfused to healthy volunteers and the platelet yield and life-span in the circulation measured [56]. This study showed that RTP had enhanced post-transfusion recovery and survival over CSP. The mechanism of reduced survival of CSP in the circulation has since been shown to be caused by clustering of GPIbα receptors on the surface of platelets and desialylation exposing β-N-acetylglucosamine (β-GlcNAc) moieties on CSP [58,59]. The exposed β-GlcNAc is recognized by Ashwell-Morell receptors on hepatic macrophages, resulting in CSP being rapidly phagocytosed and removed from the circulation [58,60].

Despite survival of RTP being shown to be longer than CSP, functionality, as measured by the ability to aggregate in response to agonists, was better preserved in CSP. It was also demonstrated that the hemostatic effectiveness of RTP was impaired, for at least 24 hours after transfusion [57,61,62] and clinical studies showed that CSP were more effective than RTP at correcting bleeding time and bleeding scores in thrombocytopenic patients, immediately upon being transfused [57,62].

Although suggestions had been made that a dual inventory should be kept by blood banks – RTP for prophylaxis and CSP for acute hemorrhage [57,63], CSP had globally all but been abandoned by the end of the 1970s, driven by the logistical challenges of managing dual inventories and due to prophylaxis for hypoproliferative thrombocytopenia outnumbering other indications for transfusion [16]. Another contributing factor may have been the platelet clumping that is seen in CSP stored in 100% plasma which is ameliorated when stored in 70% plasma to 30% platelet additive solution (PAS) [64]. However, over the past decade, there has been a renewed interest in CSP due to changes in clinical practice and a shift away from prophylactic platelet transfusions.

Along with these changes in practice, the conflicts in Iraq and Afghanistan, where there was limited access to PC due to the short shelf life, placed a greater emphasis on the importance of being able to supply PC, with the studies performed by Pidcoke [65] and Perkins [66] demonstrating the benefit of early platelet transfusion in military casualties requiring massive transfusion. As a result of these studies, a renewed interest in CSP emerged [67]. CSP may have greater hemostatic properties due to their partially activated or "primed" state as well as the potential for a longer shelf life compared to RTP. Table II summarizes the main benefits and constraints of CSP and RTP.

Attempts have been made to prevent the rapid clearance of CSP in the circulation by galactosylation of the β-GlcNAc moieties on platelets. This has been successful in murine studies but ineffective with human platelets [75].

CSP derived from apheresis platforms are currently licensed in the US by the Food and Drug Administration (FDA) for up to three days for use in the resuscitation of actively bleeding patients [76] and under an FDA exception procedure for 14 days when conventional platelet products are unavailable, or their use is not practical [77]. In addition, they have also recently been licensed in Norway, by the Norwegian Directorate of Health, as a response to the covid pandemic to mitigate the risk of blood shortages for storage up to 14 days if there was an insufficient supply of platelets [78].

Outside of hematological oncology, there is only a single published clinical trial of CSP versus RTP in complex cardiac surgery [69]. This was a pilot trial which aimed to provide preliminary data on safety and feasibility for further evaluation of the hemostatic potential of CSP. The study showed reduced chest drain output (as a means of measuring blood loss) in CSP compared to RTP and supported the feasibility of a 14-day shelf life [69].

Table II. Comparison of benefits and limitations of CSP vs RTP.

Cold Stored Platelets (CSP)	Room Temperature Platelets (RTP)
Not conductive to most bacterial growth due to low temperature, therefore reduced risk of transfusion-associated bacterial infection/transfusion-associated sepsis - bacterial monitoring methods not required.	Ideal environment for bacterial growth, leading to increased risk of bacterial contamination and sepsis [68], costly bacterial monitoring/ pathogen inactivation strategies required.
Reduced circulation time – half-life of around 1.3 days* [56] therefore unlikely to be suitable for prophylactic transfusions.	Increased circulation time (half-life of 3.9 days), reduces transfusion frequency for prophylaxis and thus risk of alloimmunization [16].
Dual inventory would be required – CSP for therapeutic transfusions and RTP for prophylactic transfusions, increasing complexity of supply chain	Single inventory for both therapeutic and prophylactic transfusions
Cheaper and easier to store - can be stored with red cell concentrates without agitation, no requirement for separate agitators and incubators.	Logistics of storage difficult - requires constant agitation to maintain gaseous exchange and aerobic respiration [23]. Requires use of bulky & expensive agitators at a regulated temperature of 22°C ± 2°C.
Can be transported in isothermal transport boxes with red cells and other medications to be used in pre-hospital treatment of trauma [16].	Requires separate transport boxes to other components. Logistics make it difficult to use in pre-hospital settings.
Potential to be stored to at least 14 days [69].	Shelf life 5–7 days (depending on bacterial screening protocol) due to platelet storage lesion and bacterial contamination risk.
PC in 100% plasma (current UK apheresis product) known to produce aggregates on cold storage	Minimal aggregate formation in RTP platelets
Significantly reduced accumulation of some pyrogenic cytokines whose presence correlates with the frequency and severity of Febrile Non Haemolytic Transfusion Reactions (FNHTRs) [70].	Higher levels of pro-inflammatory cytokines such as sCD40L and thus increased risk of FNHTRs [71].
Superior haemostatic function* [62].	Haemostatic function rapidly deteriorates throughout storage* [62].
Rapid clearance from the blood could reduce risk of thrombosis [72].	Develop a functional defect during storage which is not corrected until 24 hours post-transfusion* [62].
Preliminary data suggesting that CSP are superior to RTP in reversal of anti-platelet agents [73,74]	

*The evidence in these points was generated prior to introduction of platelet storage packs that allowed gaseous exchange

In *vitro* characteristics of CSP

Platelets rapidly change shape upon refrigeration from smooth discs into an activated spherical morphology that is defined by a loss of "swirling" and an increase in the Mean Platelet Volume (MPV) [79]. Platelets are less able to maintain their energy-dependent low cytosolic calcium levels when stored in the cold, resulting in an increase in free cytosolic calcium. The rise in calcium causes actin filament fragmentation which alongside microtubule depolymerization, results in shape change [80] that has been shown to be maintained throughout 21 days of storage [81].

Cold platelets exhibit an increased activation status, as demonstrated by an upregulation of the markers of activation such as CD62P and phosphatidylserine (PS) exposure [71]. This does not appear directly related to the activation associated with the release of α-granule contents, however, since levels of cytokines such as RANTES are significantly lower with storage in CSP compared to RTP [82]. Instead, CSP have been described as being "primed" for use [58]. Despite their increased activation marker levels, CSP have been shown to be responsive to endothelial inhibitors (nitric oxide and prostacyclin) and thus retain the ability for hemostatic control at the site of injury without causing thrombosis [83].

CSP demonstrate a superior ability to aggregate when exposed to agonists such as ADP, collagen and thrombin receptor activated peptide-6 (TRAP-6) when compared to RTP [62,84]. In addition, they have been shown by Nair and colleagues [85] to produce clots that are denser, thinner, straighter and with more branch points than RTP. All of these factors suggest that CSP are likely to have superior *in vivo* function compared to RTP.

Metabolic activity of CSP

Cold storage of platelets significantly lowers metabolic activity, resulting in a reduction in the metabolic rate of glucose consumption and lactate production through glycolysis [64]. The platelet count, pH, pO_2, pCO_2, bicarbonate and ATP concentrations are also better maintained in CSP compared to RTP [82]. Overall, this diminution of metabolic activity means that glucose remains present in CSP for up to 21 days of storage, supporting the extension of the PC shelf life [81].

Recent advances in extracellular flux (XF) analyzers have enhanced the ability to measure the bioenergetic profiles of intact cells *ex vivo*, by measuring the oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) or proton excretion of the cells [9]. This reflects the function of mitochondrial respiration and glycolysis, respectively, and unlike endpoint assays such as lactate, enables detection of metabolic function in real time, as well as avoiding isolation of mitochondria from the platelets which may lead to damage of the organelle [41]. The total ATP production rate can be measured distinguishing between the fractions of ATP that are produced from OXPHOS and from glycolysis, in addition to measuring key parameters of mitochondrial function through the use of modulators to isolate different parts of the electron transport chain (ETC).

The ETC's role is to transfer electrons through complexes I-IV in the inner mitochondria membrane (Figure 1) to the final electron acceptor, oxygen [7]. As the electrons travel through the ETC, they undergo a series of reductions. This provides the energy to drive protons against their concentration gradient into the intermembrane space, creating an electrochemical gradient comprising an increased concentration of H+ ions and positive charge distribution collectively referred to as the proton motive force. The H+ ions then flow back into the mitochondria through ATP synthase, resulting in the production of ATP [86].

The use of modulators of the ETC in the XF analyzer has the potential to provide a more precise understanding of platelet concentrate metabolism during storage through the isolation of individual parts of the ETC. The use of the inhibitor oligomycin inhibits ATP synthase, resulting in a reduction in the OCR that reflects the fraction of oxygen consumption as a result of ATP synthesis via OXPHOS. The subsequent addition of rotenone (a complex I inhibitor) and antimycin A (a complex III inhibitor) shuts down mitochondrial respiration and, with the simultaneous measurement of the acidification rate, enables the calculation of glycolysis-driven ATP production [87]. The respiratory reserve of mitochondria can be measured with the addition of a proton uncoupler, which allows protons to bypass the ATP synthase and consume oxygen at the maximum potential rate. By subtracting the basal OCR from this maximal rate, it is possible to quantify the ability of the cells to respond to a stressor with increased ATP production [88].

The XF analyzer has been used to examine the bioenergetic profiles of platelets as biomarkers for the deterioration of mitochondria in different clinical settings including cardiopulmonary bypass [89], asthma [90] and Alzheimer's Disease [91], but has had very limited use in examining bioenergetic profiles of PC for transfusion [41]. This is an area of potential future research for CSP and could provide an advanced understanding of the key role of platelet metabolism and mitochondrial health in the PSL, which is not easily discernible by the established platelet metabolic assays discussed previously (e.g., glucose consumption and lactate production).

Conclusions

The longer half-life in the circulation of RTP in comparison to CSP has allowed the supply of PC to meet the increasing demand, particularly for the management of hematology patients. However, reports of the benefits of CSP in the literature are plentiful, and together raise the question of whether routine use of CSP should be revisited. The ease of and reduced cost of storage, as well as the ease of transportation are obvious advantages when considering treatment of trauma, while the reduced risk of bacterial growth and hence longer shelf life, are key to addressing current concerns regarding blood product wastage. However, there are logistical obstacles to overcome, such as the production of aggregates when PC in 100% plasma are stored in the cold which requires a move to apheresis PC in PAS and plasma as well as the need for a dual inventory for therapeutic and prophylactic transfusion which could add to the complexity of blood supply chains.

Fully understanding the PSL that occurs at room temperature and in cold storage is critical to determining the ideal platelet product, and yet historically, examining PSL has largely been via relatively crude assessment of platelet end point functionality. Newer technology allowing for the assessment of platelet bioenergetic profiles in real-time is already being used to more accurately assess platelet activity and function in a range of disease settings, but its use in the assessment of PSL in PC is currently very limited. Application of this technology to studying mitochondrial and glycolytic function in RTP and CSP could support decisions on whether CSP may once again be introduced into blood transfusion practice for the management of bleeding.

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