Platelet Storage Temperature – How Low Can We Go?

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Key Words

Hematology · Platelet storage · Platelet transfusion · Platelets

Summary

Transfusion of human platelets, stored in blood banks as concentrates, is the treatment of choice for thrombocytopenia and bleeding. To be clinically effective, transfused platelets must circulate and retain sufficient function to recognize and react to vascular damage. Storage at room temperature is limited to 5–7 days because of the risk of bacterial growth and loss of platelet functionality, as platelets do not tolerate refrigeration. Once chilled, platelets are detected and rapidly removed from circulation despite high functionality. We recently defined a clearance mechanism for short-term (2 h) refrigerated platelets: $\alpha_M\beta_2$ receptors on hepatic macrophages recognize clustered β GlcNAc residues on platelet GPIb α receptors on refrigerated platelets. Here we review current knowledge of the mechanisms of platelet removal, the changes that occur in platelets after short and extended refrigeration, and methods to preserve platelet concentrates long-term for transfusion.

Schlüsselwörter

Hämatologie · Thrombozyten-Lagerung · Thrombozyten-Transfusion · Thrombozyten

Zusammenfassung

Die Transfusion von Humanthrombozyten, in Blutbanken als Konzentrate gelagert, sind das Mittel der Wahl für die Behandlung bei Thrombozytopenie und Hämorrhagie. Um klinisch wirksam sein zu können, müssen transfundierte Plättchen zirkulieren und eine ausreichende Funktion aufrechterhalten, um Gefäßschäden zu erkennen und darauf zu reagieren. Die Lagerung bei Raumtemperatur ist auf 5–7 Tage begrenzt wegen der Gefahr des Wachstums von Bakterien und des Funktionsverlustes; Plättchen vertragen keine Abkühlung. Einmal abgekühlte Thrombozyten werden erkannt und trotz ihrer erhaltenen Funktion sofort aus der Zirkulation entfernt. Wir haben kürzlich einen Eliminationsmechanismus für kurzzeitig (2 h) gekühlte Plättchen vorgeschlagen: Im Falle gekühlter Thrombozyten erkennen $\alpha_M\beta_2$ -Rezeptoren auf Leber-Makrophagen Cluster aus β GlcNAc-Resten der Plättchen-GPIb α -Rezeptoren. In dieser Übersichtsarbeit stellen wir den derzeitigen Kenntnisstand zur Plättchenelimination, den Veränderungen nach kurzer oder langer Abkühlung sowie den Methoden für eine Langzeithaltbarmachung von Thrombozyten zur Transfusion vor.

Introduction

Platelets maintain vascular integrity, prevent spontaneous bleeding, and work in concert with blood coagulation proteins to limit blood leakage following tissue damage. Bleeding due to thrombocytopenia is a major cause of morbidity in clinical

disorders such as sepsis, cancer, and preeclampsia. Platelet transfusion remains the sole replacement therapy for patients having active bleeding. Although the development of platelet storage techniques has permitted millions of transfusions per year, current blood banking practice for platelets is far from ideal. Platelets for transfusion are stored in gas-permeant bags

at room temperature, on large shaking racks, for ≤ 5 days, a limit imposed by the risk of bacterial growth in the bags. Other major problems associated with room temperature platelet storage are viral and bacterial contamination [1], the loss of platelet functionality [2], and the complexity of managing supply with demand. New technologies such as bacterial detection [3, 4], or pathogen inactivation [5] to reduce bacterial growth, might ultimately aid the blood banker in dealing with these potential problems. For example, current apheresis platelets have fewer significant storage lesions than products used years ago, mainly due to improvements in the materials and methods used in platelet storage and collection. Furthermore, platelet additive solutions (PAS) may prove beneficial in better preserving platelet properties during 5- and possibly 7-day storage [6–9]. Platelet refrigeration can dramatically reduce the risk of bacterial growth [10] and preserve platelet function [11, 12], thereby permitting extended storage of platelets and bolstering product inventories. However, almost 40 years ago, Murphy and Gardner [13] demonstrated that refrigerated, transfused human platelets are rapidly cleared from the circulation, showing that platelets stored at room temperature (22 °C) circulated significantly longer with a typical life span of 7–9 days versus 2–4 days for refrigerated platelets. This review addresses our current knowledge of the mechanisms of platelet removal, changes that occur in the surface of platelets at room temperature and after short and extended refrigeration, methods to preserve platelet concentrates long-term, and the necessity for robust models to study in vivo efficacy of the stored platelet product. Our studies on the mechanisms of platelet clearance following refrigeration have suggested approaches to improve the circulation of these platelets.

Room Temperature versus Refrigerated Storage

To have clinical utility, transfused platelets must both circulate and retain sufficient function such that they recognize and react to vascular damage. Clinically, the success of platelet transfusion is judged solely by an increase in the circulating platelet count. Platelet circulation in vivo is evaluated after the infusion of radiolabeled platelets that can be identified and followed with time in blood [14, 15]. However, the ability of transfused platelets to circulate in vivo, fails to provide their ‘level’ of function. At present, we lack measures for platelet function in vivo and have only a superficial understanding of factors that dictate platelet survival and/or initiate platelet clearance.

Platelet lifespan is defined by an intrinsic apoptotic mechanism [16]. Mason et al. [16] have provided evidence in mice that the pro-survival Bcl-XL protein constrains the pro-apoptotic activity of Bak to maintain platelet survival; e.g., mouse platelets lacking Bcl-XL have short survivals compared to normal, while platelets from mice engineered to lack Bak sur-

vive considerably longer. In accordance with this finding, platelets ‘aged’ in vitro to induce caspase activation are removed rapidly from the circulation [17]. Phosphatidylserine (PS) exposure is believed to be the ultimate death signal in this pathway directly initiating platelet recognition and clearance by the phagocytic system [18–21]. Storing platelets at room temperature would not be expected to slow this lifespan clock, and accordingly, activated caspase-3 levels and PS exposure increase as platelets age at room temperature [19, 22, 23]. Refrigeration of human platelet concentrates, however, prevents PS exposure [11] suggesting that it slows the intrinsic apoptotic clock and thereby would permit long-term storage. Sialylation, the addition of a terminal sialic acid to glycans, functions as an anti-clearance signal for blood cells and serum proteins, shielding the exposure of underlying immature carbohydrate epitopes such as galactose, GalNAc, and GlcNAc, ligands recognized by scavenger receptors [24–26]. For example, the sialic acid content of erythrocytes decreases as they age in blood suggesting that desialylation is a physiological trigger for clearance [25]. Also, treatment of erythrocytes with neuraminidase in vitro to enzymatically remove sialic acid unmasks penultimate β -galactose moieties and initiates erythrocyte removal through the macrophage asialoglycoprotein receptor (ASGPR) [27]. A similar mechanism may be at work to modulate platelet survival. Platelets lose sialic acid from their membrane glycoproteins as they age [28, 29], and, like erythrocytes, in vitro desialylated platelets are cleared rapidly [30, 31]. Mice deficient in the sialyltransferase ST3GalIV [32], an enzyme responsible for sialylation of certain galactose residues, have low platelet counts most likely because of accelerated platelet clearance due to their high density of exposed galactose residues. Moreover, platelets stored at room temperature progressively lose sialic acid [33]. Therefore, we conclude that carbohydrate density and/or its presentation can alter platelet survival.

Since room temperature-stored platelets remain metabolically active, they progressively lose viability and function, a phenomenon known as the ‘platelet storage lesion’ [34, 35]. Accumulation of lactate from platelet metabolism decreases the pH of concentrates, and pH levels below 6.0 are associated with loss of platelet viability. Platelet refrigeration greatly reduces lactate production [11, 12].

Platelets also slowly become activated when stored at room temperature for prolonged times [36]. The effect of refrigeration on platelet activation remains controversial. Some investigators have reported the presence of increased platelet activation markers such as exposure of surface P-selectin [11, 37, 38] following refrigeration, indicating that platelet refrigeration can induce some α -granule release. However, other investigators have reported the opposite, that refrigeration (< 21 days) retards the release of α -granule contents such as β -thromboglobulin [12, 39, 40]. Refrigerated platelets spontaneously form aggregates [41, 42], respond better to agonists than room temperature platelets [11, 43–51], and adhere bet-

ter to von Willebrand factor (vWf) under shear stress [52, 53]. Furthermore, a recent study shows that systemic hypothermia of 31–34 °C in mice accelerates thrombus formation in arterioles and venules [43], indicating that exposure to temperatures below the physiological norm may activate platelets in vivo. Critically, platelet activation per se does not induce platelet clearance since platelets treated with agonists in vitro circulate normally when transfused into primates or mice [54, 55]. Furthermore, we have demonstrated that galactosylated platelets circulate normally after refrigeration in mice, despite acquiring spiky shapes [56]. At face value, these findings appear to eliminate a central role for platelet shape change and/or P-selectin upregulation, in the clearance of stored platelets. Conversely, obtainment of a discoid shape is not a requirement for normal platelet survival, as β 1-tubulin lacking transgenic platelets, which have amorphous shapes, circulate normally [57]. In conclusion, there seems little value in assessing discoid shape of stored platelets as evidence of platelet viability in the clinical setting.

The response of platelets to hypotonic shock is another test reported to be predictive of platelet viability following storage. It measures the ability of platelets to recover from a hypotonic challenge with the notion that only ‘metabolically fit’ platelets recover. Since refrigerated platelets perform poorly in this assay [12], investigators have proposed that refrigeration impairs platelet adenosine triphosphate (ATP) production [58, 59]. It has also been reported that exposure of platelets to low temperatures can induce mitochondrial damage [60, 61] providing a mechanism for altered ATP metabolism. However, clinical studies have demonstrated clearly that refrigerated (< 2 days) platelets are hemostatically effective when transfused into humans [45, 62–67].

The major problem with storing and using refrigerated platelets remains their shortened lifespan. This phenomenon is not restricted to humans and is shared by other mammalian platelets that have been studied in baboon, rabbit, and mouse models [37, 38, 56, 68–73]. Since experimental therapy is limited by technical and ethical considerations in humans, studies in animals remain the best option for understanding this phenomenon. Historically, nonhuman primates have been used for in vivo studies of platelets. The main advantages of nonhuman primates are the similarity of their platelets to those of humans, in terms of survival times, in vitro function, and blood counts, and the fact that sufficient blood can be obtained to mimic human storage conditions [55, 74–78]. Unfortunately, studies in nonhuman primates are constrained by their high cost and limited availability. Although platelets from other mammals share the sensitivity to cold, differences in platelet half-lives and immune systems add complexity to their use. One approach that has been promoted is the transfusion of human platelets into animals, but it results in rapid platelet removal unless the animals are deprived of the bulk of their phagocytic system [69, 70, 79, 80]. For example, human platelets are tolerated in rabbits suppressed with ethyl palmitate, but the utility

of platelet survival under these conditions compared to normal is unclear. Using animals with an adaptive humanized immune system [81] could be an approach for future survival and functional studies of transfused human platelets. Dogs have been used to study fluorescently labeled rehydrated-lyophilized platelet incorporation into wounds by fluorescent microscopy [82], and the use of porcine platelets has been proposed for studies of cold-induced activation of platelets [83]. However, few in vivo platelet studies are reported in pigs [84, 85]. Murine transfusion models have been used to dissect platelet clearance mechanisms [17, 56]. Murine platelet storage is complicated because of the small volume of platelets obtainable from mice although anti-mouse platelet-specific antibodies and genetically altered mice are widely available. At present, we lack assays evaluating platelet function in vivo.

Clearance of Short-Term Refrigerated Platelets

An effort to address the clinically relevant problem of why refrigerated platelets fail to circulate led us to define a previously unsuspected, carbohydrate-dependent platelet clearance mechanism. The macrophage carbohydrate-binding $\alpha_M\beta_2$ recognizes clustered GPIb α subunits of the vWf receptor (vWfR) complex following short-term refrigeration, resulting in the phagocytosis and clearance of platelets in vivo in mice and in vitro by human THP-1 macrophages [37, 56, 86, 87]. Experiments using $\alpha_M\beta_2$ -deficient but not vWf-, complement- or P-selectin-deficient mice [54], markedly improved the survival of refrigerated platelets. Removal of GPIb α 's ligand binding-domain using the O-sialoglycoprotein endopeptidase restored the circulation of refrigerated murine wild-type platelets indicating that the external domain of GPIb α initiates clearance [37]. Subsequent work narrowed carbohydrate recognition by $\alpha_M\beta_2$ to exposed β GlcNAc (β -N-acetylglucosamine) residues on N-linked GPIb α glycans (fig. 1) [56, 86]. GPIb α -associated N-linked glycans are complex-type, branched carbohydrates covalently attached to asparagine residues. When completely assembled, they are capped by sialic acid. Removal of sialic acid (desialylation) exposes galactose and degalactosylation reveals β GlcNAc. The exposure of individual sugars is detectable by their binding to specific lectins, e.g., ricinus communis agglutinin (RCA) I binds galactose and succinylated wheat germ agglutinin (sWGA) binds β GlcNAc. Although resting platelets bind some sWGA, refrigerated platelets have markedly increased binding suggesting that altered epitope presentation of exposed β GlcNAc on GPIb α can facilitate lectin binding to refrigerated platelets. Since altered presentation correlates with a clustering of GPIb α on the surface of refrigerated platelets, we believe clustering is the cause of increased lectin recognition [37, 56]. The β GlcNAc residues recognized by $\alpha_M\beta_2$ represent either incomplete glycan processing on platelets or result from a degradative process that occurs in blood. In an attempt to remedy their poor circula-

Fig. 1. Proposed mechanisms for cold-induced platelet clearance. The von Willebrand factor receptor (vWfR) complex, specifically GPIb α , have complete (sialic acid-capped) and incomplete N-linked glycans with exposed β GlcNAc and/or galactose residues. Clustering of vWfRs and exposed β -GlcNAc initiate phagocytosis by the macrophage $\alpha_M\beta_2$ integrin. Coverage of exposed β -GlcNAc residues by galactose (galactosylation) prevents phagocytosis of short-term refrigerated platelets, but not of long-term refrigerated platelets. Extended refrigeration maybe followed by additional surface changes such as 'hyperclustering' of receptors and their associated glycans and loss of receptors and/or carbohydrate residues. This leads to phagocytosis by a $\alpha_M\beta_2$ integrin-independent mechanism, perhaps through galactose receptors and/or scavenger receptors.

tion, we galactosylated refrigerated platelets and were surprised to learn that both human and murine platelets have functional platelet galactosyltransferase(s) on their surface. These enzyme(s) respond to the simple addition of UDP-galactose by transferring galactose onto exposed β GlcNAc residues of human or mouse GPIb α [56]. Galactosylation markedly improved the survival of murine platelets refrigerated for 2 h [56]. In support of this finding, other investigators have demonstrated that platelet galactosylation prolongs the survival of long-term refrigerated human or rabbit platelets following the transfusion into rabbits [70, 71]. Subsequent experiments, however, showed that galactosylation was not beneficial in improving the circulation of 48 h-refrigerated platelets in humans [88]. While depriving the $\alpha_M\beta_2$ lectin-domain of its β GlcNAc ligand on refrigerated platelets, galactosylation theoretically provides a new ligand for asialoglycoprotein receptors (ASGPR). Hence, it was surprising that refrigerated mouse platelet circulation could be improved by

galactosylation. We postulated that the number of exposed β GlcNAc residues on GPIb α was small, such that even after clustering and galactosylation, the galactose density was insufficient to engage galactose-recognizing lectins [56]. However, this theory does not account for the recognition of long-term refrigerated human platelets by the ASGPR. Evidently, different mechanisms are involved in the clearance of short-term and long-term refrigerated platelets (fig. 1).

In summary, experiments with refrigerated mouse platelets reveal the presence of functional glycosyltransferases on the platelet surface. Glycosylation of GPIb α on the platelet surface by externally exposed glycosyl transferases influences the circulation kinetics of platelets in vivo. Hence, this work defined an important new clearance mechanism for platelets and suggested that glycan modification can be used to influence platelet circulation kinetics. These findings further indicate that carbohydrate density and/or presentation can modulate platelet survival and that platelets are unique in having func-

Fig. 2. Differential changes in platelets following short-term and long-term refrigeration. Platelets refrigerated for hours (short-term refrigeration) change from smooth discs to spiky-spheres, a process induced by F-actin cytoskeleton reorganization. Clustering of the vWf receptor complex (GPIb $_{\alpha,\beta}$ IX) $_2$ V, and N-linked glycans is initiated shortly after cooling. Prolonged platelet refrigeration (long-term refrigeration) facilitates further changes: i) Receptor and associated glycan clustering becomes maximal; ii) specific glycan residues (i.e. sialic acid and/or galactose) are removed from the platelet surface receptor(s), or receptors become cryptic due to receptor conformational changes; iii) α -granule release causing P-selectin exposure, and/or binding of plasma proteins such as fibrinogen and/or vWf to activated $\alpha_{IIb}\beta_3$ or vWf receptors.

tional glycosyltransferases [89–93] expressed on their surfaces. It is tempting to speculate that reversible glycosylation of glycans on the platelet surface is a mechanism that alters platelet survival times. It is also possible that glycosylation has a role in thrombus formation and in platelet-platelet or platelet-endothelial cell adhesion.

Differential Changes of Platelets Refrigerated for Short or Long Periods

Clustering and Conformational Changes in the vWfR Complex
Resting platelets rapidly change from discs to spidery forms at temperatures below 15 °C [94–97]. Shape change occurs within minutes following refrigeration (short-term cooling) and several investigators have suggested that increased cytosolic calcium and actin remodeling are the key initiators [95, 98, 99]. One consequence of the actin rearrangement during re-

frigeration is that surface vWfR complexes, which attach by GPIb $_{\alpha}$ to underlying filaments through filamin [100–103], are redistributed from linear arrays into aggregates [37]. Since vWfR and other receptors such as $\alpha_{IIb}\beta_3$ contain immature glycans with exposed β GlcNAc and/or galactose moieties [29, 33, 56, 89, 91, 104], refrigeration can cause profound changes in the presentation of exposed glycans on the platelet surface as we have detected using specific lectins. Glycan clustering is detected early after refrigeration [37, 56] but may increase with long-term platelet storage in plasma (fig. 2). Platelet activation induces lipid raft aggregation [105]. Recent findings indicate that platelet exposure to cold also causes lipid raft aggregation [106], indicating that not only protein-associated glycans, but also glycolipids, may cluster in the cold. It is tempting to speculate that vWfR clustering following platelet activation or refrigeration triggers lipid raft aggregation. Recent studies have revealed that refrigeration, in addition to clustering vWfR, also induces conformational changes in it.

Most investigators agree that the vWfR remains intact on the surface of refrigerated platelets, although there have been some reports of shedding [107]. These results are not incompatible, as refrigeration results in the selective loss of GPIb α binding to some monoclonal antibodies (mAbs), but not others. Some mAbs report a significant reduction in recognition of GPIb α from the platelet surface even after brief refrigeration, which further increases with time [87, 108], whereas other mAbs report no loss following refrigeration for ≤ 2 days [108]. However, some loss of surface receptors, specifically of GPIb α , due to shedding or internalization, following long-term refrigeration cannot be excluded [11] (fig. 2). Fibrinogen and vWf binding to platelets, refrigerated long-term in plasma, may also interfere with mAbs that bind to GPIb α at epitopes near the vWf binding site [108] (fig. 2).

Long-Term Platelet Refrigeration May Reveal New Insights into Platelet Clearance

Like short-term refrigerated platelets, long-term refrigerated platelets are rapidly removed in the liver following transfusion [74]. Since prolonged storage in the cold increases the binding of the galactose-specific lectin RCA I (Hoffmeister et al., unpublished), we postulate that galactose residue clustering engages a different lectin-based recognition system from the previously defined $\alpha_M\beta_2$ - β GlcNAc interaction. Specific galactose-binding receptors include the ASGPR [109], the Kupffer cell galactose receptor [110], the scavenger receptor C-type lectin (SRCL) [111, 112], and the macrophage galactose lectin (MGL) receptor (fig. 1) [113]. Since both galactose and β GlcNAc exposure would be expected to contribute to the clearance of refrigerated platelets, a combination of both galactosylation and sialylation would be required to rescue the circulation of long-term refrigerated platelets. Long-term refrigerated platelet clearance might also be stimulated by platelet-associated fibrinogen, which would be recognized by the α_M I-domain on macrophages. However, fibrinogen also binds to the surface of activated platelets which circulate normally.

Additional studies are needed to determine the exact clearance mechanism of long-term refrigerated platelets. Considering the complexity of the changes induced by long-term platelet refrigeration, whether glycosylation (i.e. platelet sialylation) alone will be sufficient to overcome the rapid clearance of long-term refrigerated platelets awaits testing.

Platelet Cryopreservation and Freeze-Drying

Freeze-drying of platelets has been considered as one alternative for long-term preservation of platelets. Since the pioneering work of Klein et al. [114] in 1956, a concerted effort has been made to apply the process of freeze-drying to generate

a stable platelet-based infusible hemostatic agent. Some progress has been reported in preparing lyophilized platelets that preserve platelet integrity and some functionality. One approach to preserve cell integrity applies fixation with aldehydes or similar cross-linking agents [82, 115]. However, fixation by various cross-linking agents compromises platelet function, particularly secondary hemostatic mechanisms such as fibrinogen binding [115] and might be expected to generate neo-antigens. Fixed and lyophilized platelets have been reported to retain sufficient hemostatic function to stop bleeding [115] in thrombocytopenic animal models [116]. Clinical trials of this freeze-dried platelet product will reveal within the next few years whether or not it has useful application for transfusion on a daily basis.

Alternatively, Crowe et al. [117–119] have proposed the use of antifreeze compounds to protect freeze-dried platelet preparations. Evidence has been presented that one of these compounds, trehalose, maintains membrane microdomain organization in refrigerated and freeze-dried platelets. Platelet membranes have been shown to undergo lateral phase separation during prolonged storage in the cold [105, 120] causing certain surface receptors to be selectively and irreversibly incorporated into detergent resistant membrane domains. Trehalose treatment prevents this phenomenon. Once again, clinical trials are required in order to determine if this technology has feasibility for platelet transfusion practice.

Another approach for achieving long-term stabilization of platelets is long-term freezing in dimethylsulfoxide (DMSO), a cryopreservative [121–123]. Currently approved methods for platelet preservation are labor intensive and require high DMSO concentrations (5–6%) as cryoprotectant [124]. Not surprisingly, platelet counts and functional activity decrease after freezing and thawing, and treated platelets require excessive washing to reduce the DMSO toxicity [125, 126]. 2 studies have reported that autologous platelet concentrates cryopreserved at -180°C in 2% DMSO and ThromboSol[®] (LifeCell Corp., The Woodlands, TX, USA), a platelet storage additive consisting of selected second messenger effectors (amiloride, sodium nitroprusside and adenosine), diminish platelet activation related to cooling [127]. Cryopreserved and ThromboSol-treated platelets sufficiently increased platelet counts when transfused into severely thrombocytopenic patients, indicating that freeze-dried platelets are viable to a certain extent [128, 129].

It remains an important concern in the development of infusion therapeutics for hemostasis that the product is both effective at inducing coagulation at wound sites and safe with respect to pathologic intravascular coagulation and no development of neo-antigens. Despite some promising clinical results, the problems of significant loss of cell number, optimal freeze rates, freezing temperature, resuspension medium and substantial impairment of in vitro functional capacity are obstacles that have yet to be overcome. There is no in vitro test which can predict platelet viability and hemostatic efficacy in

vivo with certainty [130, 131]. Clinical trials are required to obtain information if any of the above proposed methods to cryo-preserve platelets are applicable for transfusion routine practices.

Conclusions

The reason that platelet concentrates are stored at room temperature in blood banks is because once refrigerated, platelets do not circulate. Room temperature storage results in a progressive loss of platelet functionality. Although it is widely believed that loss of discoid shape in the cold is the cause of poor survival, shape change has no adverse effects on platelet circulation kinetics or hemostatic function, and, in fact, refrigerated platelets actually retain high functionality. In an attempt to understand why refrigerated platelets circulate poorly, we defined a carbohydrate-based platelet clearance mechanism. The macrophage phagocytic integrin, $\alpha_M\beta_2$, recognizes GPIb α -associated β GlcNAc moieties clustered following short-term refrigeration (2 h), causing the ingestion and removal of platelets in vivo [37, 56]. In an attempt to remedy this problem, we found that platelets have surface-exposed galactosyl-transferase(s) that cover β GlcNAc residues with galactose when provided with the UDP-galactose substrate. Galactosylation restores the circulation of refrigerated mouse platelets in vivo and prevents the phagocytosis of refrigerated human

platelets in vitro [56]. However, it does not prevent the removal of human platelets refrigerated long-term in plasma [88]. Investigations of the relationship between vWfR clustering/conformational changes following long-term platelet refrigeration indicate that prolonged platelet refrigeration induces further profound rearrangements of platelet surface receptors and carbohydrates compared to short-term refrigeration. These changes bring additional phagocytic mechanisms into play, that cooperate with the $\alpha_M\beta_2$ receptor to remove long-term refrigerated platelets. We speculate that a combination of sialylation and galactosylation, achievable by addition of sugar substrates alone, may remedy the survival of long-term refrigerated platelets. However, a detailed analysis of the surface changes induced by long-term platelet refrigeration and their relationship to clearance mechanism(s) is required.

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