Chapter

Cryoprotection of Platelets by Grafted Polymers

Mark D. Scott, Nobu Nakane and Elisabeth Maurer-Spurej

Abstract

Unlike red blood cells (RBC) which are stored at 4°C, platelets are stored at 22–24°C (room temperature) due to biophysical and biochemical changes induced by cold temperatures aggregately known as the 'cold storage lesion' (CSL). However, 22°C storage greatly increases the risk of microbial growth, thus limiting the safe storage of platelets to only 5–7 days (versus 42 days for RBC). Consequent to the short shelf life of platelets, blood services face chronic shortages of these life-saving cells. To overcome both the risk of microbial contamination and the constrained supplies of platelets, renewed research into attenuating the CSL and/or determining where cold stored platelets are clinically suitable are ongoing. In this chapter, we show that the covalent grafting of methoxypolyethylene glycol (mPEG), a biocompatible polymer, to the membrane of platelets attenuates the CSL. Moreover, the grafted mPEG serves as a potent cryoprotectant allowing platelets to be stored at 4°C, or frozen at −20°C, while retaining normal platelet counts and biologic function. The successful development of platelet PEGylation may provide a means by which the cold storage of platelets can be achieved with a minimal loss of platelet quality while improving both platelet microbial safety and inventory.

Keywords: cryopreservation, cryoprotection, platelets, blood banking, cold storage, PEGylation, immunocamouflage, methoxypoly(ethylene glycol), polymer, aggregation

1. Introduction

Platelet adhesion and aggregation at the site of vascular injury are key events required for normal vascular homeostasis and wound repair. [1–4] Platelets are produced from megakaryocytes in the bone marrow and, while lacking a nucleus, contain a number of specialized granules such as alpha-granules and dense granules. Normal, resting platelets have a discoid morphology which changes upon activation to 'spiny spheres' arising from the formation of pseudopodia. This shape change coincides with the rearrangement of the actin cytoskeleton. Upon activation, platelets adhere to the subendothelium at sites of vascular injury, aggregate and initiate coagulation to stop bleeding (*i.e.* haemostasis).

Consequent to this essential role, platelet transfusions have evolved as a crucial therapeutic tool in the treatment of a large number of diverse clinical conditions including acute bleeding, surgery, treatment of a variety of cancers, patients with platelet abnormalities and autoimmune diseases such as Idiopathic Thrombocytopenic Purpura (ITP) [5]. To meet the increasing clinical needs, blood systems within developed countries produce in excess of 5,000,000 transfusion

doses annually [6]. However, demand for platelets continues to increase annually while the rate of blood/platelet donations are actually declining leading to an inventory that is chronically constrained [7].

The constraint of platelet inventory is in large part due to an inability to safely store platelet products for greater than 5–7 days. Historically, platelets, like red blood cells (RBC), were stored at 4°C and successfully used clinically. However, multiple studies from the late 1960s to the early 1970s demonstrated that 4°C (*i.e.* cold) storage of platelets resulted in significantly reduced *in vivo* survival times compared to platelets stored at 22°C (warm storage) or endogenously produced platelets (2–4 vs. 7–9 vs. 10–12 days, respectively) [8–17]. The observed loss of *in vivo* viability and *in vitro* morphology and function was termed the platelet cold storage lesion (CSL) and resulted in the change in standard blood banking practice to storing platelets at 22–24°C by the early 1970s.

The CSL is multi-dimensional and is best characterized as the sum of all the deleterious changes in platelet morphology, biochemistry and function that arise from the time the blood is withdrawn to the time the cold-stored platelets are transfused. The CSL is characterized, in part, by loss of discoid shape (*i.e.* abnormal morphological forms), decreased mean platelet volume, increased size

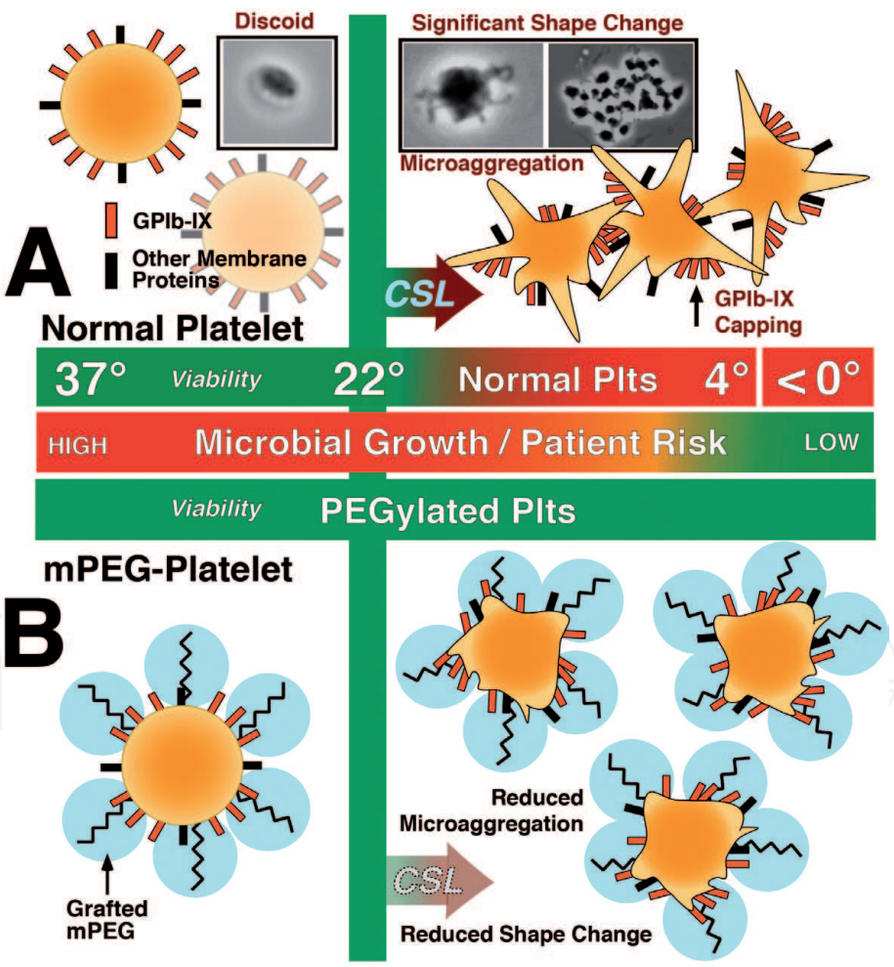

Figure 1.
Schematic view of the prevention of the platelet 'cold storage lesion' (CSL) by membrane PEGylation. Panel A: The normal discoid shape of platelets is lost as platelets are cooled below ~18°C. As a consequence of cooling, pseudopodia formation occurs leading to microaggregation of platelets (photo insert). Additionally, membrane proteins such as GP1b-IX aggregate on the surface. These changes lead to both mechanical and immunological clearance from the circulation. Panel B: PEGylation of platelets reduces both the shape change (e.g. *fewer pseudopodia) and prevents microaggregation of the cold stored platelets. Consequent to the attenuation of the CSL lesions by the grafted mPEG, platelets can be stored for extended periods (> 7 days) at 4 or −80°C thereby improving platelet inventory and supply management while reducing the platelet discard rates. As noted, cold storage also significantly reduces the risk of microbial growth thus, potentially, improving transfusion safety.*

heterogeneity, pseudopodia formation, increased release of platelet α-granules and cytosolic proteins, altered surface protein expression (*e.g.*, glycoproteins such as GP1b-IX), increased procoagulant activity, aggregate formation, and reduced platelet counts—all of which are also characteristic of platelet activation (**Figure 1A**) [8–16, 18, 19]. In contrast, warm storage of platelets maintained platelet morphology, activation potential and greatly improved *in vivo* circulation times [8–16].

However, the warm storage of platelets was not without risk as it was demonstrated that warm storage significantly increased the risk for bacterial growth should bacteria be introduced to the platelet unit during collection [20–24]. Indeed, numerous North American screening studies have indicated that approximately 1/3500 platelet units (primarily platelet rich plasma; PRP) are bacterially contaminated posing a potential hazard to already at-risk patients [23, 25, 26]. Consequent to this risk, multiple blood systems have implemented costly universal bacteriologic screening of donor platelets. Hence, development of new technologies to improve both platelet safety and inventories will be crucial in meeting the ever increasing demand for platelet products.

2. What was 'OLD' is 'NEW' again

Consequent to the clinical demand and supply chain issues, several studies over the last several years have re-explored the potential use of 'cold-stored' platelets. Initial excitement regarding cold-stored platelets arose in 2003, Hoffmeister et al. investigated the mechanism(s) underlying the CSL and experimentally demonstrated that the shape change alone induced by cold storage itself did not result in poor platelet survival in a murine model [18, 19]. Instead, Hoffmeister et al. hypothesized that poor platelet survival resulted from an irreversible membrane clustering of alpha subunits of glycoprotein Ib (GPIbα). Their studies reported that exposed, terminal, beta-linked N-acetylglucosamine (βGlcNAc) residues on clustered GPIbα were recognized by the lectin domain of type 3 complement receptors (CR3; $\alpha_M\beta2$; CD11b/CD18) on liver and splenic macrophages. This immunorecognition resulted in the rapid clearance of cold stored donor platelets via phagocytosis. Hoffmeister also demonstrate that phagocytosis of briefly chilled murine platelets could be inhibited and *in vivo* survival prolonged by enzymatically galactosylating the terminal βGlcNAc residues on GPIbα. These findings led them to propose that enzymatically masking the exposed ßGlcNAc residues on the N-glycans of the clustered GPIbα molecules by galactosylation would allow for the cold storage of human platelets without adversely affecting platelet function. However, enthusiasm for glycosylated platelets subsided when subsequent studies by Wandall et al. demonstrated that galactosylation alone did NOT protect murine or human platelets from prolonged cold storage (*e.g.* >48 hours) [27].

More recently, the 'old' (1960) has become 'new' (2019) as transfusion scientists have begun to reexamine the clinical utility of platelets stored at 4°C. Indeed, the original 1960's/70's studies that initially discovered the platelet CSL, also reported that 'cold-stored' platelets were still effective *in vivo* in preventing acute blood loss. Hence, current clinical studies are investigating the use of 4°C stored platelets for at least some transfusion demands [28–45]. In general, recent studies suggest that, while these 'old technology' cold-stored platelets could be of benefit for acute haemostatic transfusion needs, cold stored platelets still exhibit morphological changes and poor *in vivo* survival making them unlikely candidates for chronic replacement therapy in patients with already accelerated platelet clearance or as therapeutics for patients with cancer or who have undergone bone marrow transplantation.

3. Hypothesis: attenuating the CSL via membrane-grafted mPEG

Consequent to our earlier work on polymer grafting to intact cells (*e.g.* RBC, lymphocytes), we hypothesized that the polymer induced immunocamouflage of platelet membranes with methoxypoly(ethylene glycol) [mPEG] could prevent or circumvent the immune recognition of cold stored platelets [46–49]. This hypothesis was supported by our previous studies on RBC and leukocytes (White blood cells; WBC) that demonstrated that the grafted polymer prevented cell:cell interactions (*e.g.* RBC Rouleaux formation; Phagocytosis of opsonized RBC; and Lymphocyte:APC) and membrane protein clustering (RBC CR1 aggregation) of the mPEG-modified cells while maintaining normal cellular function [50–71]. Hence, it was hypothesized that mPEG-grafting to platelets would prevent platelet aggregation and the clustering of GPIbα, and phagocytic recognition of the transfused mPEG-platelets (**Figure 1B**). Moreover, because soluble mPEG is a known cryoprotectant, we hypothesized that the grafted polymer might also attenuate other 'cold-induced' mechanical lesions of the CSL induced by cold temperatures and even freezing of donor platelets [72–79]. Indeed, the ability to freeze and recover donor platelets would both greatly increase platelet inventory and potentially expand the use of platelet therapy to geographic locations where platelet therapy is not commonly practiced.

4. Polymer engineering of platelets

All human experiments were done in accordance with the approval of the University of British Columbia Clinical Research Ethics Board and the Canadian Blood Services Ethics Review Board in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Following informed consent, fresh platelet rich plasma (PRP) and, in some cases, buffy coat platelets (the standard of care in Canada and Western Europe) were obtained from volunteer donors or the Canadian Blood Services Network Centre for Applied Development (NetCAD) Laboratory (Vancouver, BC). PRP samples are similar to the platelet preparations used clinically and have an advantage of a lower level of manipulation (*e.g.* centrifugation) than buffy coat or apheresis platelets. Care was taken to assure adequate representation of males and females and no individuals were excluded on the basis of age (within acceptable age range of donation of 17–71) or race.

Based on our previous studies, platelets were PEGylated using a semi-automated PEGylation device to maintain a constant platelet:polymer ratio **(Figure 2)** in a micromixing chamber to assure uniform polymer grafting [50, 52, 56, 71]. Platelets were modified with monofunctional (*i.e.* one binding site per chain) mPEG-succinimidyl propionate (SPA-mPEG). Previous studies (not shown) within our laboratories determined that the optimal molecular weights for the PEGylation of platelets were 2–5 kDa which were used in these studies. For comparison of the effects of different linker chemistry, some studies simultaneously examined mPEG-benzotriazolyl carbonate (BTC-mPEG). Both SPA- and BTC-mPEG (Laysan Bio, Inc., Arab, AL, USA) react with protein lysine residues and covalently attach via formation of a stable amide bond (**Figure 2C**). To demonstrate the efficacy of the polymer-mediated grafting, the mPEG-mediated immunocamouflage of platelet CD9 was assessed by flow cytometry. CD9 is a constitutive tetraspan membrane glycoprotein present on resting platelets that modulates cell adhesion and migration.

Control and PEGylated mini-units (approximately 50 ml/unit; ~500 × 10^9/L) of platelets were stored at 4 or 22°C with agitation per Canadian Blood Services standard operating procedures. Storage at −80°C was done separately in the sample

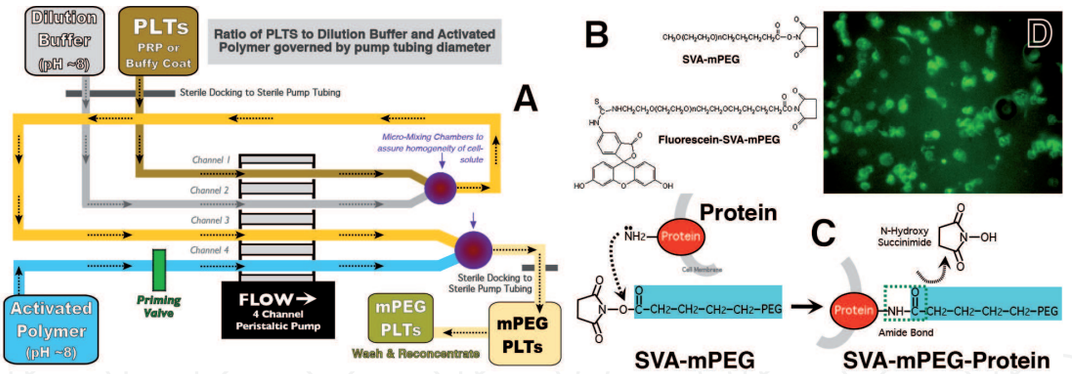

Figure 2.
Production of mPEG-platelets and the SVAmPEG reaction scheme and grafting efficacy. Panel A: semi-automated PEGylation device allowing for control of the mPEG:platelet ratio for uniform grafting levels [71]. Panels B and C: the structure (B) and reaction scheme (C) for activated SVA-mPEG. The SVA-linker chemistry forms a stable amide bond with platelet membrane proteins. Panel D: Fluorescein-conjugated SVA-mPEG demonstrated that platelets were uniformly modified using our semi-automated methodology.

mini-unit blood banking bags. Storage was done for up to 12 days under the prescribed conditions (note: normal storage at 22°C is only allowed for 7 days). Storage bags were sampled aseptically in biosafety cabinets; washing and lysis procedures were performed as described previously [80–82]. Platelet counts were determined using an Advia 120 Hematology Analyzer (Bayer Inc., Toronto, Canada).

5. Effect of 4°C storage on mPEG-platelets

The covalent grafting of mPEG to PRP platelets resulted in the efficient immunocamouflage of CD9 (**Figure 3A**). As demonstrated in **Figure 3A**, virtually 100% of control platelets were $CD9^+$, while the mPEG grafting to the platelets exhibited dose effect on the immunocamouflage of CD9. More importantly, the grafted polymer significantly decreased the aggregation of human platelets at 4°C. As microscopically demonstrated in **Figure 3B**, temperature exerted a significant effect on the morphology and microaggregation of control PRP preparations. As anticipated, minimal differences were observed in the control platelets at 37° (*i.e. in vivo* conditions) versus 22°C (normal *in vitro* storage temperature). Importantly, PEGylation with either SCmPEG5000 or BTCmPEG5000 yielded platelets with comparable morphology to the control cells at 37 and 22°C. However, upon thermal transition from 22 to 4°C, control platelets were observed to form significant microaggregates characteristic of the CSL. In stark contrast, neither the SCmPEG5000 nor BTCmPEG5000 modified platelets exhibited any significant microaggregation consequent to the mPEG-mediated inhibition of cell:cell interaction [66, 68]. Morphological analysis of the SCmPEG5000 and BTCmPEG5000 modified platelets suggested that SCmPEG5000 better prevented cold induced shape change relative to the BTC polymer resulting in the SC-linker chemistry being further explored.

The mPEG-mediated inhibition of cold-induced platelet aggregation was also not a short term effect. As demonstrated in **Figure 4**, unmodified control platelets demonstrated significant shape change, microaggregation, and a dramatic (~30%) decrease in platelet count. In contrast, minimal microaggregation was noted in the PEGylated samples following 12 days storage at 4°C. PEGylated platelets also retained a more discoid shape (though some pseudopod formation was noted). Due to the inhibition of microaggregation and inhibition of activation induced shape change, the mPEG-grafted platelets also resulted in a significantly improved platelet count.

Importantly, PEGylated platelets were functionally normal as evidenced by their *in vitro* aggregation response to thrombin. As shown in **Figure 5A**, phase contrast microscopy of washed control and PEGylated platelets resuspended in normal plasma both maintained a smooth, resting morphology. However, in response to 2 IU/mL thrombin activation (**Figure 5B**), both control (a) and PEGylated (b; 10 mM SCmPEG-5000) platelets fully aggregated at 37°C (1000 rpm stir speed) in an aggregometer (Chronolog, Havertown, PA, USA). This normal aggregation of PEGylated platelets occurred despite the significant immunocamouflage (see below) of the platelet membrane surface. Moreover, as shown in **Figure 5C**, phase contrast microscopy demonstrated that control and PEGylated platelets form microscopically very similar thrombin-induced clots. Interestingly, if PEGylated platelets were suspended in PEGylated plasma essentially no *in vitro* aggregation was observed, likely due to the PEGylation of plasma proteins involved in

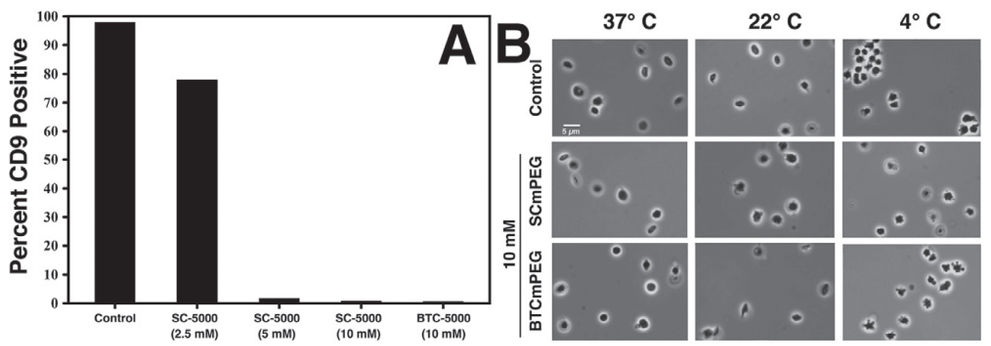

Figure 3.
Immunocamouflage of platelets by grafted mPEG. Panel A: CD9 is effectively camouflaged by grafted 5 kDa polymers of SC- and BTC-activated mPEG. The efficacy of immunocamouflage was a function of mPEG grafting concentration. Panel B: While both SC- and BTC-mPEG demonstrated similar efficiency in camouflaging CD9, photomicrographs showed that the SCmPEG better preserved platelet morphology at 4°C. SCmPEG was consequently used for all further studies.

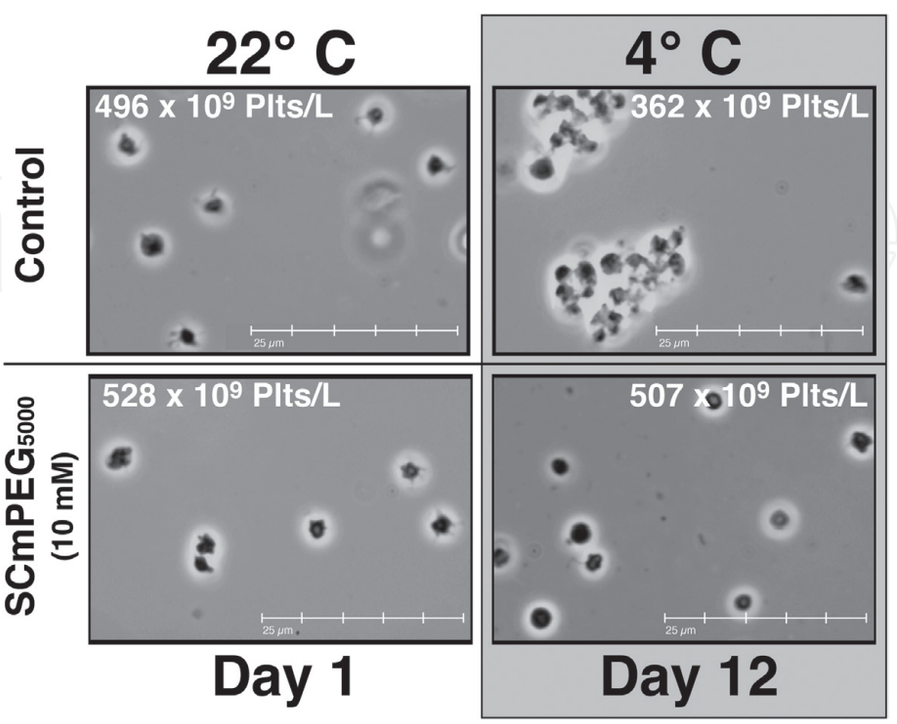

Figure 4.
PEGylation inhibited 4°C cold-induced platelets aggregation and shape change. The improved viability of the cells is accompanied by maintenance of the pre-storage platelet count (day 0).

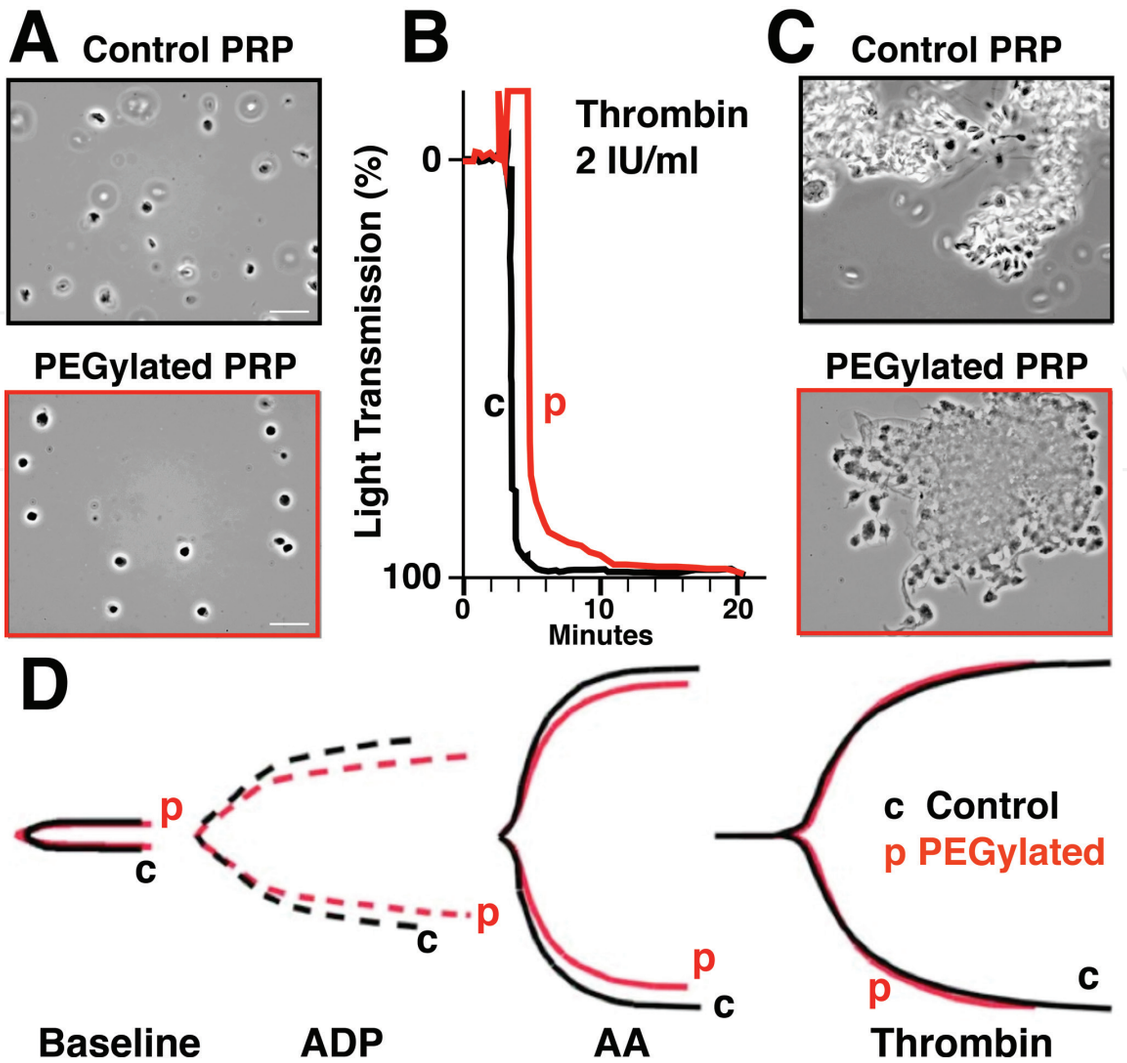

Figure 5.
PEGylated platelets are functional and aggregate in vitro *in response to agonists. Panel A: Phase contrast microscopy of control and PEGylated PRP platelets in plasma demonstrate that both populations maintain a smooth, resting morphology. Panel B: Aggregometer analysis of control (c) and PEGylated (p) platelets demonstrate normal responses to 2 IU/mL thrombin (37°C; 1000 rpm). Panel C: Control and PEGylated PRP platelets form microscopically very similar thrombin-induced clots. Panel D: PEGylation did not affect platelet thromboelastography (TEG) as denoted by the virtually identical TEG tracings. These results demonstrated that PEGylated platelets participated normally in the in vitro clotting assay. Platelet mapping with TEG determines total platelet function. The two symmetric arms show the same results. Shown are representative responses of control (c; black lines) and PEGylated (red lines; SCmPEG5000) platelets at rest (baseline) and in response to ADP, AA, and thrombin activation.*

clot formation. However, these data indicated that PEGylated platelets would be functional when transfused into an actively bleeding patient regardless of whether they were stored in the presence of PEGylated plasma, normal plasma or a platelet storage solution as their functionality was restored in the presence of normal plasma. Thromboelastographic (Haemonetics, Braintree, MA) analysis of control and PEGylated platelets further demonstrated the normality of polymer modified platelets in response to multiple platelet agonists. As noted in **Figure 5D**, control and PEGylated platelets demonstrated virtually identical results when exposed to adenosine diphosphate (ADP), arachidonic acid (AA) or thrombin activation indicating that clot formation should not be adversely affected by PEGylation—especially since in most circumstances PEGylated donor platelets will represent ~50% or, most typically, much less of the platelets in a clot.

Clinically, visual inspection for 'swirl' may be the only pre-transfusion 'quality' test of the platelet unit—though even this is rarely done. The swirl test is a

noninvasive method that literally works by swirling the bag and looking for light diffraction (*i.e.* refractiveness) [83–86]. Due to the discoid shape of resting (unactivated) platelets, light is diffracted creating a cloud- or swirl-like appearance of the bag. Platelet activation causes a disc to sphere morphology change where upon orientation dependent changes in light diffraction are no longer observed. A dull platelet bag is deemed 'activated' while a refractive bag is 'resting'. Unsurprisingly, despite the low cost (*i.e.* free) of the swirl test, it actually tells very little as to the quality of the platelet unit. Over the last few years, a new technology has been developed to quantitatively measure platelet quality using dynamic light scattering. The ThromboLUX (LightIntegra Technology, Vancouver, BC) quantitatively measures platelet morphology (shape change) and temperature response and provides a quantitative replacement to the qualitative and subjective 'platelet swirl' [62, 83–85, 87–92]. Mechanistically the ThromboLUX utilizes dynamic light scattering to examine a small volume (~30 μl) of platelets to quantitatively assess platelet size and morphological changes arising from temperature cycling (37 to 20 to 37°C). The ThomboLUX generates a Dynamic Light Scattering (DLS) value that correlates with platelet activation status. Moreover, the ThromboLUX is capable of quantitating the number of platelet-derived microparticles and evidence of microbial contamination. The ThromboLUX technology has been clinically validated and provides a correlation between the DLS score and a patients corrected count increment at 24 hours (CCI24) post transfusion [84]. Shown in **Figure 6A** is the ThromboLUX

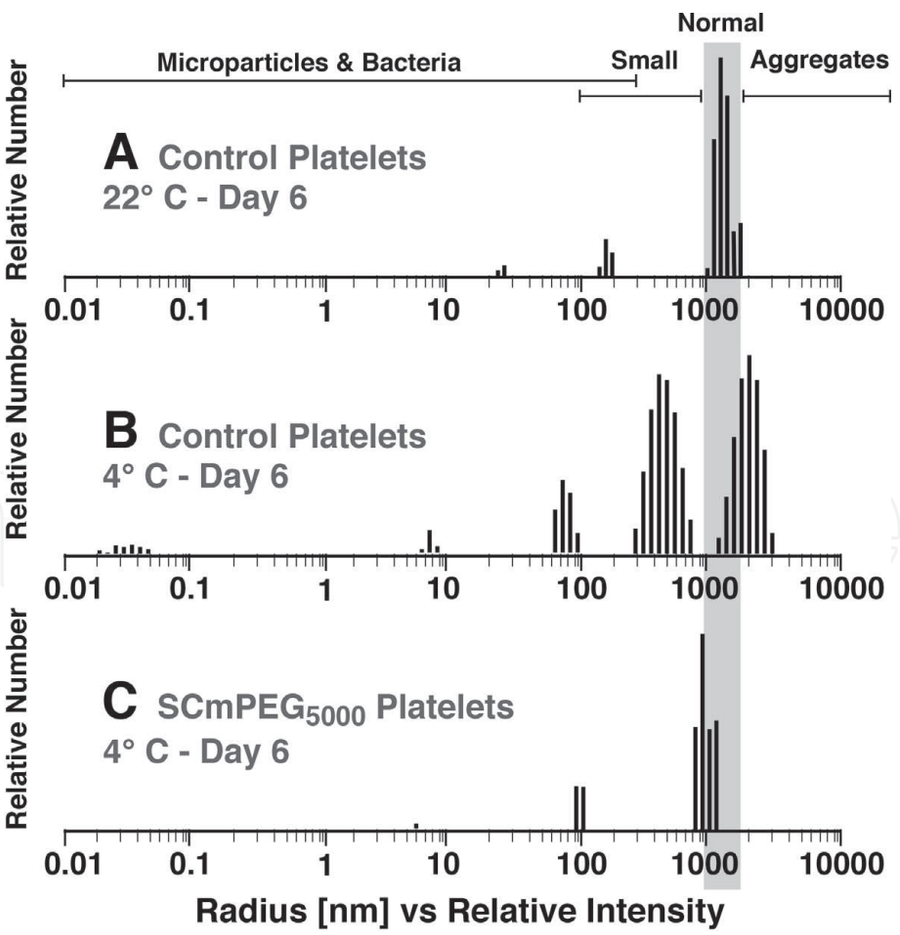

Figure 6.
ThromboLUX dynamic light scattering analysis of platelets following 6 days storage. Panel A: Control platelets stored at 22°C. panel B: Control platelets stored at 4°C. panel C: ScmPEG5000 (10 mM) platelets stored at 4°C. As shown, mPEG grafting resulted in significant cryoprotection. The Y-axis is particle count while the x-axis reflects the hydrodynamic radius distribution. All samples were prepared from the same donor platelet unit.

profile of control platelets stored 6 days at 22°C. However, cold storage of the same platelet preparation resulted in a dramatic shift of the platelet peak (**Figure 6B**) with the appearance of microparticles, smaller platelets and platelet aggregates. In contrast, cold stored (6 days) PEGylated platelets (10 mM, 5 kDa) yielded a DLS profile similar to the 22°C stored platelets with no evidence of aggregate formation and minimal microparticle formation (**Figure 6C**).

6. Effect of −80°C storage on mPEG-platelets

To further assess the cryoprotective effects of the grafted mPEG polymer, freezing studies were conducted on the control and SCmPEG platelets. While previous work on PEG as a cryoprotectant utilized a soluble form, work with PEG and other cryoprotectants (DMSO and Trehalose) demonstrated that the primary site of protection was at the level of the cell membrane [72–79]. As shown in **Figure 7**, following 12 days storage at −80°C, covalently bound SCmPEG provided significant cryoprotection as reflected by both platelet morphology and improved cell counts. This finding is in stark contrast to control platelets which exhibited significant fragmentation and dramatically reduced cell counts post storage and thawing.

The covalent grafted mPEG exerted additional benefits post thaw. As shown in **Figure 8**, SCmPEG-grafted platelets exhibited improved morphology, and less fragmentation, immediately post-thaw when compared to control cells. Indeed, the grafted polymer provided comparable (or better) cryoprotection than DMSO. Moreover, following washing and re-concentration of the freeze-thaw

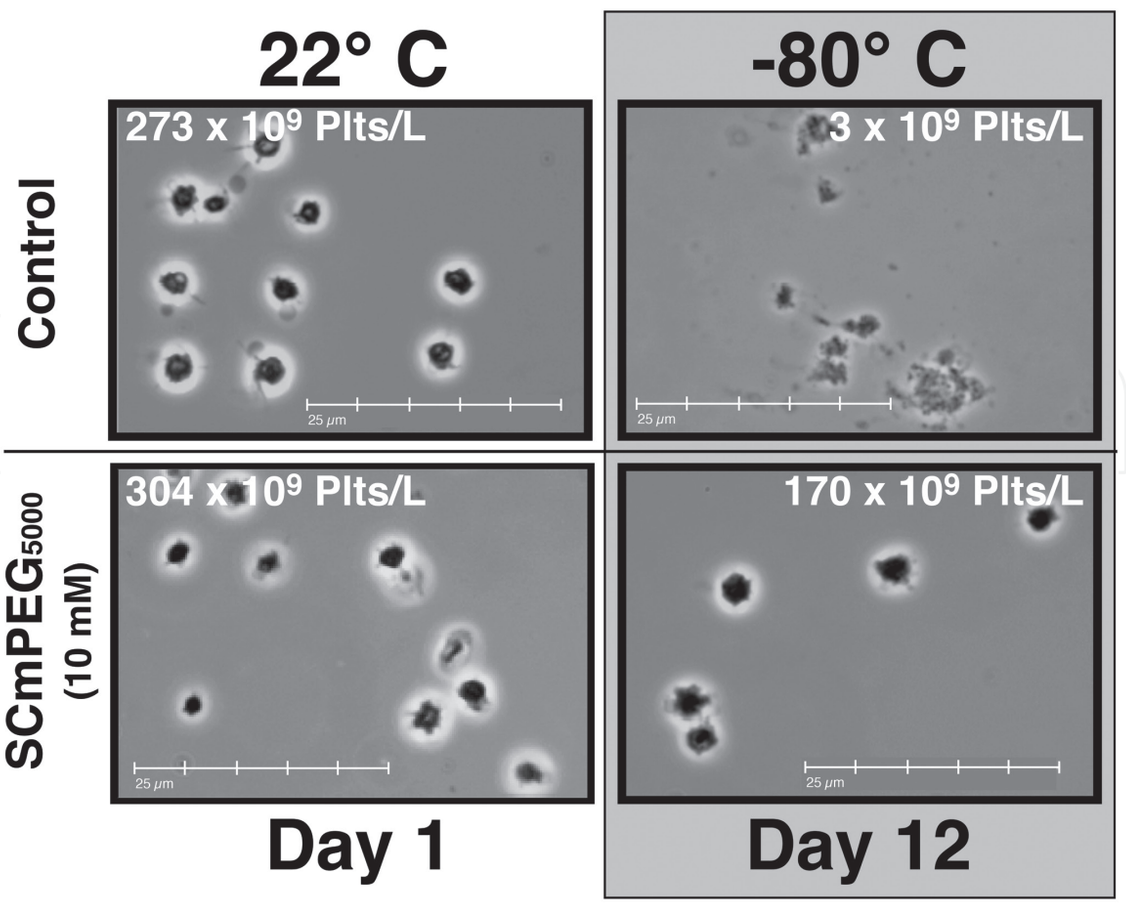

Figure 7.
PEGylation inhibited −80°C cold-induced platelets aggregation and shape change. The improved viability of the cells is accompanied by significantly improved maintenance of the pre-storage platelet count (day 0).

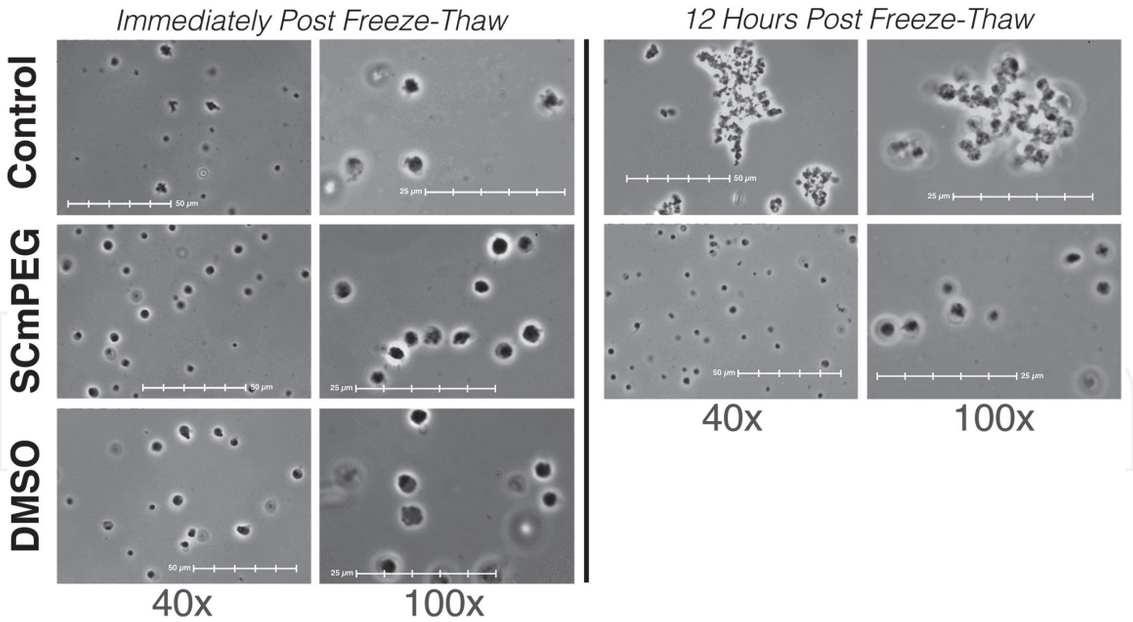

Figure 8.
PEGylation gives rise to significant cryoprotection from freeze-thaw injury. As shown, after 12 days of storage at −80°C, unmodified control platelets exhibited significant platelet destruction, loss of morphology, and a significant ($p < 0.001$) decrease in platelet count. In contrast, subsequent to freeze-thaw, PEGylated platelets demonstrated normal discoid morphology and few detectable microaggregates and were very comparable to platelets cryopreserved with DMSO, the standard cryoprotectant for frozen RBC and platelets. Moreover, unlike control platelets, the PEGylated sample did not form microaggregates when incubated at 22°C.

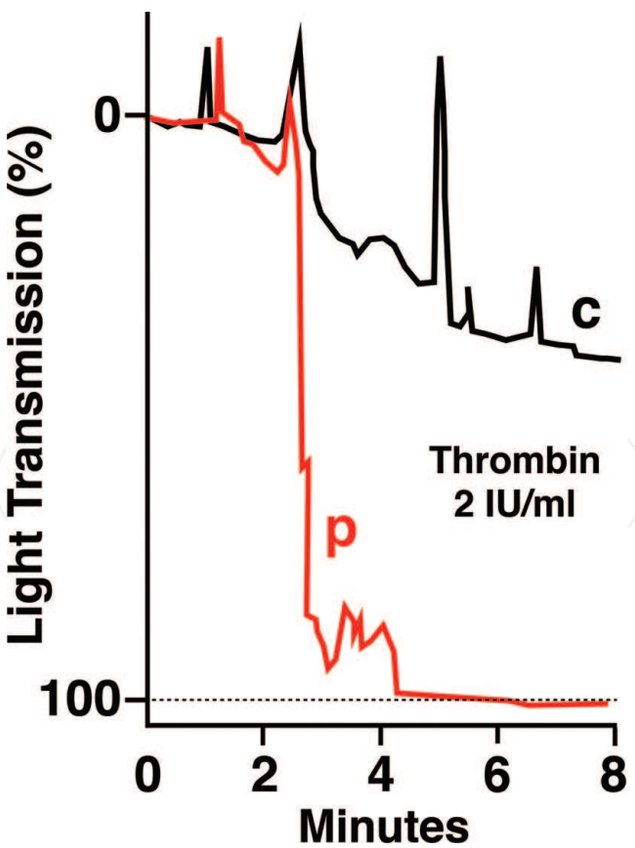

Figure 9.
Post storage at −80°C, the aggregation of control and SCmPEG platelets was assessed in response to thrombin (2 IU/mL). As shown, thawed control platelets exhibited limited aggregation (~40% light transmittance) after 8 minutes. In contrast, the PEGylated platelets demonstrated robust aggregation (100% light transmittance at ~4 minutes). Shown are representative responses of control (black lines) and PEGylated (red lines; SCmPEG5000) platelets.

control and SCmPEG platelets, control platelets demonstrated significant aggregation when stored at 22°C overnight (12 hours). In contrast, the SCmPEG-platelets demonstrated no aggregation over the same 12 hour time frame.

While the maintenance of morphology and platelet numbers post −80 storage was promising, the key question was whether these platelets were functional. To assess platelet function, thrombin (2 IU/ml) induced aggregation was assessed. As shown in **Figure 9**, thawed control platelets exhibited a poor response to thrombin (see **Figure 5B** for a normal response) as demonstrated by very limited aggregation. Moreover, the aggregation of the control platelets was very slow as seen by the slope of the aggregation curve. In contrast, the thawed SCmPEG platelets demonstrated significant, and rapid, thrombin mediated aggregation. Indeed, near maximal aggregation was achieved within approximately 3 minutes and very closely resembled the thrombin activation curves of fresh control and SCmPEG PRP (see **Figure 5B**).

7. Discussion

Platelet transfusions are a critical component in the treatment of both traumatic acute injury and a number of chronic diseases. However, unlike RBC which are stored at 4°C, platelets are stored at 22–24°C (room temperature) due to the induction of the CSL at temperatures below ~18°C. While the CSL encompasses a multitude of biophysical and biochemical changes, perhaps the most apparent effect is the production of platelet aggregates. To prevent the CSL, blood services worldwide have successfully stored platelets at 22°C. However, warm storage has its own risks as it greatly increases the risk for microbial growth limiting the safe storage of platelets to only 5–7 days (versus 42 days for RBC) and the outdating of a significant number of donor units. Consequent to the short shelf life of platelets, blood services face chronic shortages of these life-saving cells. To overcome both the risk of microbial contamination and the constrained supplies of platelets, renewed research into attenuating the CSL and/or determining where cold stored platelets are clinically suitable are ongoing.

To circumvent the microbial risk, and improve platelet inventory, our research has examined the potential use of cold stored, mPEG-grafted, platelets. As demonstrated by our *in vitro* experimental findings, the covalent grafting of mPEG to donor platelets significantly reduced the severity of the 4°C CSL while maintaining normal haemostatic function. This was evidenced by the maintenance of 'normal' platelet morphology, the lack of microaggregation, and normal platelet activation by thrombin and normal aggregation as determined by thromboelastography and aggregation studies. Moreover, due to the cryoprotective effects of the grafted mPEG, polymer-modified platelets could be stored at −80°C and thawed and still retained normal morphology and haemostatic function. In contrast, the freezing of normal platelets in the absence of a cryoprotectant (*e.g.* DMSO) resulted in significant cellular damage resulting in vastly reduced recovery, loss of haemostatic function and subsequent microaggregation when incubated at room temperature for 12 hours.

Interestingly, while not a focus of this chapter, polymer size (*e.g.* 2, 5 or 20 kDa) was an important factor when considering the cryoprotection of platelets while maintaining the unique functions of the platelet. In contrast to RBC and WBC in which longer chain polymers (*e.g.* 20 kDa) were optimal, short chain polymers (*e.g.* 2–5 kDa) were optimal for platelet cryopreservation. However, the disparity between RBC and WBC relative to platelets is not as unanticipated as it appears.

The goal of RBC and WBC PEGylation is to induce immunocamouflage (*i.e.* prevent immune recognition) and, especially for WBC, prevent cell:cell communication (*e.g.* allorecognition). Clearly for platelets, cell:cell interaction is crucial for their haemostatic function. Hence, the primary goal of platelet PEGylation is to prevent thermal injury to the membrane while maintaining normal platelet function. While all sizes of the grafted polymer could prevent cryogenic injury, long chain polymers inhibited platelet activation and their subsequent aggregation. In contrast, the short 2–5 kDa polymers provided adequate cryoprotective effects while allowing for normal agonist-driven activation.

8. Conclusions

PEGylation of donor platelets with short chain (2–5 kDa) mPEG effectively prevent the overt morphological changes arising from the CSL. Moreover, the polymer-grafted platelets retained their normal haemostatic function following both cold storage (4°C) and freezing (−80°C) as evidenced by thromboelastography and aggregation studies. Importantly, cold storage of platelets would improve transfusion safety as it would diminish the risk of microbial growth in a blood product destined for use in at risk patients. Also of potential clinical and economic importance was the finding that mPEG-grafted platelets withstood freezing in the absence of other cryoprotectants such as DMSO. The use of frozen platelets, requiring no DMSO removal step, could expand the availability of platelet transfusions to geographic regions in which they are not currently available or where donor recruitment or production facilities do not exist. The successful implementation of this technology for the cold storage of platelets would be of significant benefit to transfusion recipients by increasing the availability of platelets for transfusion.

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Conflict of interest

The Canadian Blood Services (Ottawa, ON, Canada) has patents relating to the cold storage of platelets [48, 49]. MDS, NN and EMS are inventors cited on said patents.

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