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Frozen Platelets—Development and Future Directions

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ABSTRACT

Storage requirements and outdated of platelets represent a continued challenge for blood banks. These hurdles are confounded for rural area hospitals or in military deployments. Over 60 years of research and development into frozen platelets have generated a stable and reproducible product. Valeri's method to freeze platelets in 6% dimethyl sulfoxide (DMSO) and storage at -80°C allows for long-term storage alleviating burdens placed on blood banks. Clinical studies show that frozen platelet transfusions are safe with no related thrombotic or other serious adverse events. There are ongoing efforts to demonstrate cryopreserved platelet (CPP) superiority in efficacy studies designed in trauma or cardiac surgery patients. Technical advances in CPP manufacturing including closed system manufacturing, applications of pathogen reduction technology and potency standard characterization add to the appeal of CPP as an alternative to traditional liquid-stored platelets (LP) in settings of supply shortages, mass casualty, active bleeding, rapid provision of HLA-compatible platelets, and remote care.

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Abbreviations: AIHA, autoimmune hemolytic anemia; APU, apheresis platelet unit; cGMP, current good manufacturing practice; CPP, cryopreserved platelet; DMAC, dimethylacetamide; DMSO, dimethyl sulfoxide; DoD, Department of Defense; FDA, Food and Drug Administration; FFP, fresh-frozen plasma; GPIb α , glycoprotein 1b alpha; HLA, human leukocyte antigen; ITP, idiopathic thrombocytopenic purpura; ISBT, International Society for Blood Transfusion; LP, liquid platelet; NBRL, Naval Blood Research Laboratory; PAS, platelets in additive solution; PR, pathogen reduction; ROTEM, rotational thromboelastometry; RT, room temperature; TGA, thrombin generation assay; TEG, thromboelastography; TF, tissue factor; TPH, thrombin peak height; WHO, world health organization.

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Frozen Platelet History

Platelet storage requirements and outdated are continued challenges for blood banks motivating research into alternative storage methods including lyophilized (freeze-dried) and cryopreserved (frozen) platelet products. The use of frozen platelets in a clinical setting dates to 1956 where platelet concentrates were frozen in plasma and used to treat thrombocytopenic 2 to 15-year-old children. The results were encouraging with transitory hemostasis in 62% of transfusions and no thrombotic or serious adverse events observed [1]. Stefanini and Kistner (1958) [2] also displayed hemostatic effects following transfusion of frozen platelets; however, this study did not find maintenance of circulating platelet concentration. In the 1960s to 70s, clinicians emphasized sustained post-transfusion peripheral blood platelet concentration driven by the prophylactic use of platelets in thrombocytopenic cancer patients. This priority was evidenced following the work of Murphy and Gardner that supported room temperature (RT) storage of platelets versus the standard of care practice of refrigerated storage [3-5].

Among the challenges with freezing cells is mitigating the formation of intracellular ice crystals that severely decrease cell viability post-thaw. Cryobiological research identified a variety of additive cocktails to preserve platelet integrity and viability including various sugars, dimethylacetamide (DMAC), dimethyl sulfoxide (DMSO) and glycerol [6-13]. In animal models, cryoprotected frozen platelets generated circulating post transfusion recoveries at day-1 ranging from 30% to 87% [6-8,12]. Early clinical studies had day 1 recoveries around 30% [9,10]. Still prominent in these reports is the emphasis on maintaining platelet circulation as an outcome above any other post transfusion benefit.

In the 70s and early 80s, Charles Schiffer developed a program to stock-pile autologous frozen platelets for alloimmunized leukemia patients refractory to donor platelets [14-17]. From 1976-1981, over 150 patients received more than 700 frozen platelet transfusions [17]. The summation of 5 years of clinical data showed frozen platelets are safe and effective with approximately 50% post-transfusion recovery and reduced bleeding time compared to liquid-stored platelets in chemotherapy induced thrombocytopenia patients [17].

Simultaneously, Robert Valeri at the Naval Blood Research Laboratory (NBRL) continued work on cryopreservation of platelets optimizing the addition of 6% DMSO followed by -80°C storage. Frozen platelets are then thawed and undergo a post-thaw wash to remove the DMSO [18,19]. Valeri found post-thaw platelets present with a partially activated phenotype in vitro [20-22]. Clinical studies revealed Valeri's 6% DMSO platelets were superior to traditional RT stored platelets at reducing blood loss in cardiopulmonary bypass surgery with no adverse thrombotic events [23]. Valeri's group continued to advance the cryopreservation technique by removing excess DMSO before freezing [24]. The 'no-wash' method decreased storage volume and streamlined post-thaw processing and is the foundation of most current CPP development work.

Current Key Manufacturing Points

Decades of research have generated a stable and reproducible frozen platelet product. Currently, our program processes an apheresis platelet unit (APU) under 2 days post-collection with the Valeri no-wash method to manufacture CPP. Removal of most of the DMSO before -80°C storage yields a final CPP volume that is 10-15% of the starting volume, i.e. a standard APU of ~ 275 mL produces approximately 25 mL of CPP with a platelet concentration of $\sim 6.5 \times 10^6$ platelets/ μL . Good manufacturing practice (cGMP) protocols consistent with blood banking practices are utilized. Labeling practice conforms to the International Society for Blood Transfusion (ISBT) standards in accordance with AABB (formerly American Association of Blood Banks) and the US Food and Drug Administration (FDA) standards and permits unit tracking throughout the products' history. Products are stable when stored in a

mechanical freezer for 2 years. As long-term storage is a motive for CPP research and development, we have recently shown product stability can be extended from 2 to over 5 years at -80°C (Supplementary Material 4).

Characterization, Safety, and Efficacy of CPP

Recovery of frozen/thawed CPP results in a loss of approximately 25% of the initial yield [24]. When compared to conventional RT liquid-stored platelets, CPP are characterized by acquisition of a spherical shape with decreased ability to swirl [25]. In general, CPP have an activated phenotype characterized by increased surface expression of P-selectin [26] and phosphatidylserine [27]. Two types of platelets are rapidly appreciated in CPP products [27] (Fig. 1). The primary group (75%) of CPP is comprised of platelets with activated morphology characterized by loss of discoid shape, increased pseudopodia (Fig. 1 arrows) and presence of centralized granules (Fig. 1 arrowheads). The other 25% consists of post-secretion platelets depleted of granules and lacking intracellular architecture (Fig. 1 asterisks) as well as loss of GPIIb/IIIa expression. In addition, microscopic analyses of CPP identify large amounts of microparticles of varied size, different sedimentation rates and granule content [27,28] and hemostatic potential [29].

The aforementioned phenotype, along with the presence of microparticles, suggests that CPP activation state may result in pro-clotting effects (desired or undesired). In vitro functional tests have not been successfully linked to the clinical efficacy and/or safety of CPP. Aggregation response to agonists and adhesion of CPP to collagen are reduced [27,28,30], but fibrin polymerization by CPP/plasma seems to be conserved [30]. Time kinetics of thrombin generation demonstrates a larger thrombin peak height (TPH) with shortened reaction lag time for CPP compared to LP [31]. However, the endogenous thrombin potential of CPP is only slightly higher than LP [31], suggesting that while the pre-activated state may result in rapid thrombin generation, the overall thrombin generation capacity of CPP is similar to LP. Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) also indicate faster clot initiation for CPP than liquid-stored platelets [30] with a clot firmness half of that reached by LP [32].

In the last few years, there have been significant advances in our understanding of the safety and efficacy of CPP. Classical in vivo radionuclide-labeled platelet kinetic studies (a.k.a. recovery and survival) in healthy research subjects have demonstrated that the 24 hour-recovery of CPP is approximately 50% of LP with only mildly ($\sim 15\%$) decreased survival, suggesting again that at least two cohorts of platelets with different in vivo survival behavior are contained in CPP [27,33]. These results are also supported by data from autologous and allogeneic CPP infusions in patients in which there is an $\sim 50\%$ lower 1-hour platelet count increment compared to allogeneic LP [17,34].

Several non-controlled, observational studies of CPP in bleeding patients have been reported, but with limited ability to define outcome superiority or inferiority. As noted above, Schiffer and his group began transfusing CPP into highly alloimmunized thrombocytopenic patients after chemotherapy with encouraging results [17]. One of the largest of these studies to date was reported by the Dutch Army in support of North Atlantic Treaty Organization troops [35]. The Dutch Army's experience in Afghanistan from 2006 to 2010, with over 1100 CPP products transfused, indicates that the use of frozen components is not only safe, but also may result in better survival in combat casualties [35]. In this study the effect of the introduction of a massive transfusion protocol which incorporated frozen red cells, plasma and platelets in combination with liquid red blood cells was evaluated for frozen products compared to retrospective patient data. In the group of patients who required massive transfusion, there was statistically significant improvement in survival (56% vs 86% after introduction of the protocol). Additionally, for patients not requiring massive transfusion an

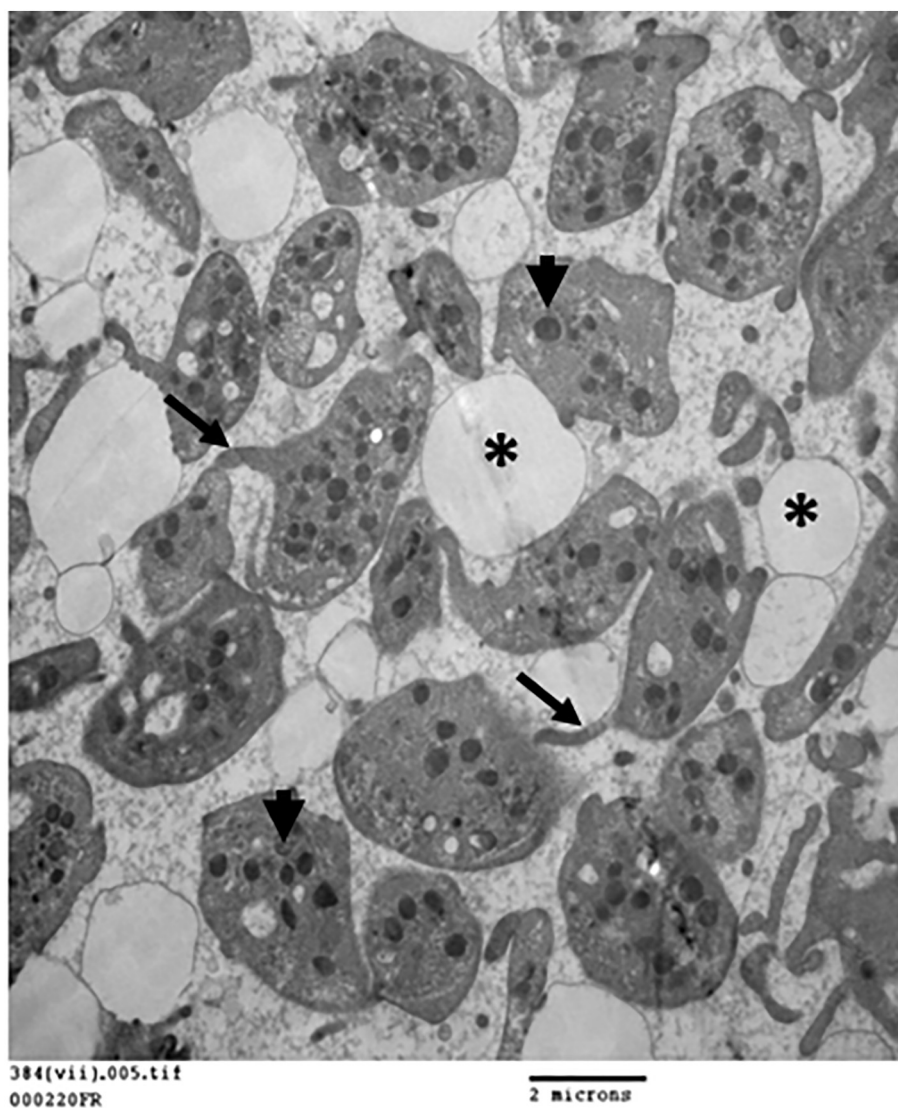


Fig. 1. Transmission electron microscopy of CPP Showing 2 Types of Platelets Post-Thaw. Transmission electron micrograph at 7000 \times of CPP. Platelets with pseudopodia (arrows) and centralized granules (arrowheads) comprise most cells. A second type of platelet is found lacking intracellular architecture (asterisks) in CPP post-thaw.

improvement was also noted (87.3% vs 96.1%) but this did not reach statistical significance.

Some controlled studies for safety and/or efficacy have also been reported, although with limited power and numbers. A careful safety study was performed in bleeding (WHO grades II-IV), thrombocytopenic, hematology/oncology patients. In this multicenter, dose-escalation study, 28 patients were randomized to receive up to 3 units of CPP. There were no serious adverse events reported, but unfortunately this trial was not designed to compare efficacy [33]. Valeri's group [36] showed that autologous CPP were functionally competent when transfused into healthy subjects given aspirin and reduced their bleeding time prolonged by the aspirin intake. Bohonek and colleagues [37], in an unblinded randomized clinical trial, found that CPP products were safe when administered to polytraumatized and massively bleeding patients. Lower platelet counts were observed in CPP recipients (median: $41.5 \times 10^9/L$) compared to those receiving LP (median: $97.0 \times 10^9/L$) when hemostatic control was attained with no difference in any of the clinical or laboratory outcomes. The underpowered nature of this trial did not reach relevant conclusions on efficacy. The Cryopreserved vs Liquid Platelet (CLIP)-I trial, a double-blind multicenter feasibility and safety clinical trial in 121 patients undergoing cardiac surgery found no significant difference in blood loss (medians of 715 mL in CCP recipients vs 805 mL in LP recipients, at 24 hours, with a 90 mL difference

— 95% confidence interval, -343.8 to 163.8 mL, $P = .41$), or red cell usage (3 units in the CPP group vs 4 units in the LP group, difference 1 unit —95% confidence interval: -3.1 - 1.1 units; $P = .23$) and no serious adverse events associated with the infusion of up to 3 units of CPP [38]. Unfortunately, this latter study was underpowered and the CPP group received a larger amount of fresh-frozen plasma (FFP) than the control group, not allowing to draw a conclusion regarding the efficacy of CPP vs LP.

The only study that has demonstrated superiority of CPP in a prospective, randomized phase II clinical trial was reported by Khuri and colleagues in 1999. They observed that cardiopulmonary bypass surgery patients treated with CPP received significantly fewer platelet units, had significantly lower median postoperative blood loss and were transfused with fewer total blood components, without serious adverse events [23]; but no significant differences in intraoperative blood loss. Blood loss after heparin reversal with protamine was lower in patients transfused with CPP than in patients transfused with LP (median of 1721 mL vs 2298 mL respectively; $P = .007$). Analysis of blood loss after exclusion from the LP group of patients who had the longest duration of cardiopulmonary bypass surgery remained significantly higher in the patients receiving the LP transfusions (median 2329 mL vs 1830 mL in the patients receiving CPP, $P = .0089$). However, several caveats in the experimental design of this trial mostly related with the

methodology used to calculate blood loss hamper a correct interpretation of the results. Ongoing efforts are currently being supported by the United States Department of Defense (DoD), the Australian Government and other Governments of NATO countries [39] to demonstrate superiority in efficacy studies designed in trauma or cardiac surgery patients.

Key Developments and Evidence Necessary for Future Success

Pathogen Reduction

Blood products such as CPP are expected to reside in strategic inventory stocks for several years. The emergence of new or modified pathogens during that period could cast doubt on inventory safety if targeted screening tests were not in place during stock preparation. In the face of a new pathogen, the effectiveness of pathogen reduction (PR) methods could be rapidly verified without retesting inventory, and thus acquit the stock of associated transfusion transmitted disease risk. In addition, PR treatment would be expected to further reduce the remote chance of septic transfusion reactions associated with transfusion transmitted bacteria.

There are currently three PR methods developed for treatment of platelets: INTERCEPT (Amotosalen, UV-B; Cerus Corporation; Concord, CA, USA), Mirasol (Riboflavin/UV-A, UV-B; TerumoBCT, Lakewood, CO, USA) and the THERAFLEX UV-Platelets system (UV-C, MacoPharma, Tourcoing, France). The concept of cryopreservation of PR-treated platelets has been highlighted in two review articles [40,41], yet has only been evaluated at a cursory, *in vitro* feasibility level for two of the three currently-available PR technologies. Meinke et al. [42] examined PR-CPP prepared from pooled buffy coat derived platelet concentrates treated with the amotosalen/UVA system prior to cryopreservation. The cryopreservation process was in 5% DMSO but in a unique configuration of a low freezing volume. Post-thaw products were resuspended in fresh plasma prior to testing. The PR-CPP appeared to be more susceptible to freeze/thaw damage than the untreated CPP especially evident in observations of platelet ultrastructure by transmission electron microscopy. *In vitro* hemostatic function was maintained compared to CPP for clot formation in the ROTEM. Six et al. [43] also evaluated amotosalen/UVA photochemical treatment and cryopreservation in 6% DMSO in a small *in vitro* feasibility study. They observed PR-CPP had reduced response to TRAP6 yet an increased TPH compared to CPP controls. The authors felt these changes were small and may not have practical effects on the overall function of PR-CPP. Johnson's team in Australia examined the UVC PR method effect on CPP [44]. Pooled buffy coat derived platelets in additive solution (PAS) were cryopreserved with 5–6% DMSO and resuspended in plasma following thawing. They observed a tendency for the PR-CPP to be more susceptible to freeze/thaw damage and activation which was exacerbated during a post-thaw storage period of 24 hours.

A major gap in the development and effective long-term deployment of CPP is the evaluation of the effect of PR platelets cryopreserved in DMSO. Additional preclinical work is indicated for evaluation of all PR methods on all methods and iterations of CPP preparation. *In vitro* data may be adequate bridging studies for comparison to untreated CPP, however *in vivo* kinetic studies of circulating recovery and survival, as well as efficacy studies in bleeding patients may be indicated.

Closed Manufacturing and Processing Systems

Manufacturing and prepare-for-transfusion methods that have been reported have not maintained a closed system through all manufacturing and post-thaw processing. Closed systems eliminate the risk of extraneous bacterial contamination of blood products during handling and manipulations, thus reducing the overall risk of septic transfusion reactions. The original manufacturing process developed by Valeri and employed by others employed DMSO preparations in glass vials [27].

This necessitated open operations only properly performed in an ISO Class 5 environment following applicable regulations for filling of pharmaceutical products [45]. Such methods are burdensome and expensive. Recently, 27% DMSO in sterile-dockable, DMSO-compatible bag systems have been introduced permitting closed system preparation of CPP; thus, permitting manufacturing in a standard blood bank laboratory without extra environmental controls. Preparation for transfusion of CPP post-thaw has necessitated use of open systems for addition of resuspension solutions such as PAS, saline or plasma, requiring transfusion of the product within 4 to 6 hours. This could result in product loss if the clinical logistics do not permit immediate product transfusion. Development of closed systems for post-thaw handling would increase the shelf life of the prepared CPP up to several days and facilitate use in a variety of clinical settings.

CPP Potency

Biological products are required to have adequate potency to effect a given result when administered to a patient, i.e., therapeutic effect. Potency tests may be *in vitro* and/or *in vivo* tests specifically designed for a product [46,47]. The potency for cellular blood components has classically been determined by volume and cell content (eg, hemoglobin or platelet concentration) to result in a targeted clinical result following administration of a standardized dose (eg, increase in patient Hb of 1 g/dL or platelet concentration by 80 000 platelets/ μ L) [48], whereas other biologics have activity or potency specified in units of activity per volume or mass. The FDA has directed CPP developers to select and develop a potency test that is specific to CPP. In the setting of active bleeding, it is believed that support of thrombin generation in the patient may be the primary mode of action of CPP. Therefore, in consultation with FDA, we selected an *in vitro* thrombin generation assay (TGA) to develop and apply for characterization and description of the specific activity of CPP as the potency test. The test and approach that follows has been shown to distinguish between types of products (APU vs CPP) as well as between individual unit preparations of these products. The applicability and interpretation of this *in vitro* potency assay remains to be shown as predictive of therapeutic effect when CPP is administered to patients.

The Calibrated Automated Thrombogram (CAT; Thrombinoscope BV, Maastricht, Netherlands) uses fluorescence to measure thrombin generation over time as the substrate ZGGR-aminomethylcoumarin (AMC) is cleaved by thrombin; thereby, releasing the AMC chromophore. The CAT assay employs a thrombin calibrator, a modified non-clot forming thrombin (α 2-macroglobulin-thrombin) that produces thrombin-like activity that is used to correct for inner filter effect, quenching variation among individual plasmas, and substrate depletion. Using this platform, the potency assay for CPP has been defined with Octaplas (Octapharma USA Inc, Hoboken, NJ) as the suspending media to avoid variation in reaction inhibitors present in FFP, tissue factor (TF) concentration of 1.0pM, and 3 targeted platelet concentrations in the reaction well of 10, 20, and 44×10^3 plt/ μ L within the log-linear response range of the assay (Supplementary Material 1 and 2). To facilitate generalization and accuracy of reporting, the CAT internal calibrator reagent has been calibrated against the WHO international thrombin standard (Supplementary Material 3). Outcome parameters of TPH and endogenous thrombin potential (ETP) are converted from nM and nM*min to IU/L and IU/L*min, respectively. TPH corrected to IU-thrombin/L has been selected as the primary outcome. An internal Potency Standard (PS) is prepared from a pool of CPP prior to freezing then frozen as 1 mL aliquots at $\leq -65^\circ\text{C}$. PS aliquots are thawed and resuspended in a manner to replicate full CPP unit methods. One dilution series of PS is assayed in each TGA plate along with test products. The Potency outcome parameter is determined from the TPH at the midpoint (approximately 20×10^3 plt/ μ L) divided by the platelet concentration in the test well to give IU-thrombin/million platelets.

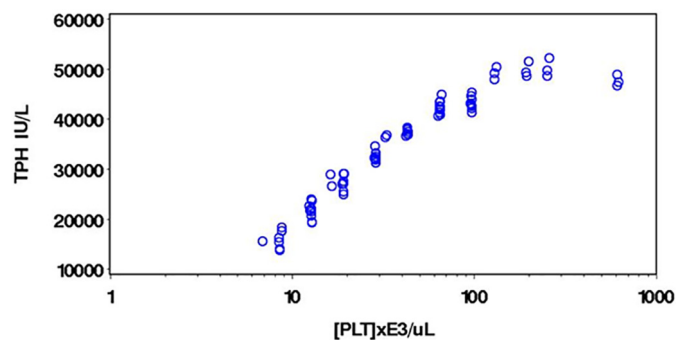


Fig. 2. Dose Response of Potency Assay for CPP. TPH corrected to WHO-IU has a log-linear platelet-dose response from $7\text{--}50 \times 10^3$ platelets/ μL with $R^2 = 0.96$ for platelet concentrations $<50 \times 10^3$ platelets/ μL .

The validation approach for the CAT is taken from the ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Test and Methodology Q2(R1) [49]. The TGA outcome is proportional to the concentration of CPP and APU, and linearity (log-linear) response ranges are $7\text{--}96 \times 10^3$ plt/ μL for APU and $7\text{--}44 \times 10^3$ plt/ μL for CPP (Fig. 2). The assay is precise within the log-linear response range for CPP (CV <9%); however, less so for APU with a CV of 24.2%. Thrombin generation is inhibited in a dose dependent manner by blocking phosphatidylserine with lactadherin, thus demonstrating the specificity of the TGA response to thrombin [50].

Potency results of 4 CPP cohorts with average storage times at $\leq -65^\circ\text{C}$ from 270 days (9 months) to 1971 days (5.4 years) compared to 5-day plasma stored APU are shown in Table 1 (Supplementary Material 4 for additional in vitro characteristic of 4 and 5 year stored CPP). CPP potency reported as in vitro thrombin generation potential is 6.7 times greater than APU. How this potency parameter translates to clinical therapeutic effect remains to be shown in future clinical trials.

Applications of CPP

Supply Shortages

Availability of fresh platelet concentrates, both apheresis and whole blood derived, are affected by a short shelf life, relatively high outdate rates (above 10% for 5-day platelets though less than 5% for 7-day platelets) and frequent shortages. Product shortages are managed on the level of the transfusion service where shortages promote decreasing the transfusable dose and delaying or denying prophylactic transfusions. Shortages of platelet concentrates create an ethical dilemma for transfusion medicine specialists. CPP is a potential solution to this problem. Applications of CPP (Fig. 3) include a ready inventory of ABO typed, low isoagglutinin titer CPP units (with or without PR) that can be stored and prepared in a similar fashion to FFP or cryoprecipitate which have been handled by transfusion services for decades. CPP can be easily

used during shortages and provide for significantly better support for bleeding patients. An important change in the current transfusion practice would refer to triggers for transfusion. CPP will be best suited for bleeding patients while fresh units could be retained for prophylactic transfusions in the outpatient setting.

Response to Mass Casualty Events

As the timing of a mass casualty event is unpredictable and injuries may vary greatly between victims, the blood demand can vary greatly. A review of recent (1980–2020) mass casualty events with the use of more than 50 RBCs per event underscores a complex relationship between the size of the event and the actual blood use [51]. The review highlights increased utilization of plasma such that the observed ratio of plasma/RBC/platelets was six, four and one-half units per admission. This new observation also underscores increased demand for plasma and platelets. The author of this review noted that in some recent mass shootings up to 42 units of platelets were used, significantly more than available in inventory at many trauma centers. CPP would be an ideal on shelf component and its long shelf life and timely availability could help in management of victims.

A small study illustrates the feasibility of using CPP instead of fresh apheresis product. In this study patients who suffered from trauma or massive bleeding received either CPP (25 patients) or APU (21 patients) [37]. The clinical outcomes in both groups were comparable though, expectedly, the platelet count post-transfusion was higher in the group receiving APU. These observed results require additional larger, well designed, randomized controlled trials in this patient population.

Platelet Support in Remote Locations (Rural Civilian, Remote Military)

Frozen blood components can be particularly useful in situations where blood support is required far away from the hospital, such as military operations and rural/remote locations. There is growing evidence that the availability of blood components close to the location of injury improves overall survival. As noted for the Netherlands Armed Forces, deployment of frozen components, including platelets, has clearly demonstrated the practicality and effectiveness of CPP in remote locations [35,52].

Bleeding Patients (Surgical, ED, GI, Other Medical)

As noted above, shortages of platelets may impede our ability to resuscitate actively bleeding patients. In such patient populations a product which has a better hemostatic characteristic would be desirable. CPP does provide additional prothrombotic activity such that patients suffering from massive hemorrhage due to surgical or gastrointestinal bleeding may achieve hemostasis faster than patients receiving fresh liquid-stored platelets. The results of a well-designed dose escalation study reported by Slichter support this hypothesis [33]. Though not designed to compare efficacy, 58% of patients who received CPP had improved

Table 1

In vitro potency for CPP. The thrombin generation assay is used to define CPP potency with tissue factor (TF) concentration of 1.0pM in Octaplas. Calculated potency values for a platelet concentration of 20×10^3 plt/ μL are shown for CPP stored up to 5 years and fresh apheresis platelet controls. [$\bar{x} \pm \text{SD}$ (minimum – maximum)]

Study cohort	N	CPP time in freezer (days)	TGA potency at 20 k (IU/ 10^6 plt)	Platelet conc. ($\times 1000/\mu\text{L}$)	Volume (mL)	Total platelets ($\times 10^{11}$)
CPP 18–7-A	30	1971 \pm 78	1.72 \pm 0.17	5145 \pm 645	53.9 \pm 1.7	2.8 \pm 0.3
5 y		(1825–2057)	(1.30–2.00)	(3638–6730)	(51.1–57.7)	(2.1–3.6)
CPP 18–7-A	29	1639 \pm 75	1.65 \pm 0.13	5429 \pm 671	53.4 \pm 1.9	2.9 \pm 0.4
4 y		(1552–1757)	(1.41–1.92)	(4437–7088)	(50.2–58.5)	(2.3–4.1)
CPP 18–3-A	24	270 \pm 232	1.53 \pm 0.13	4139 \pm 518	54.5 \pm 3.4	2.2 \pm 0.2
<2 y		(13–727)	(1.29–1.85)	(2893–5354)	(48.9–60.0)	(1.7–2.7)
CPP 17–12-D	63	422 \pm 3.5	1.58 \pm 0.17	4433 \pm 396	55.2 \pm 2.1	2.4 \pm 0.2
<2 y		(413–430)	(1.22–2.14)	(3728–5320)	(50.7–59.9)	(1.9–2.9)
Apheresis Platelet	24	na	0.24 \pm 0.06	1192 \pm 151	289.5 \pm 32.7	3.4 \pm 0.3
18–3-A			(0.15–0.37)	(938–1645)	(223.0–348.4)	(2.8–4.0)

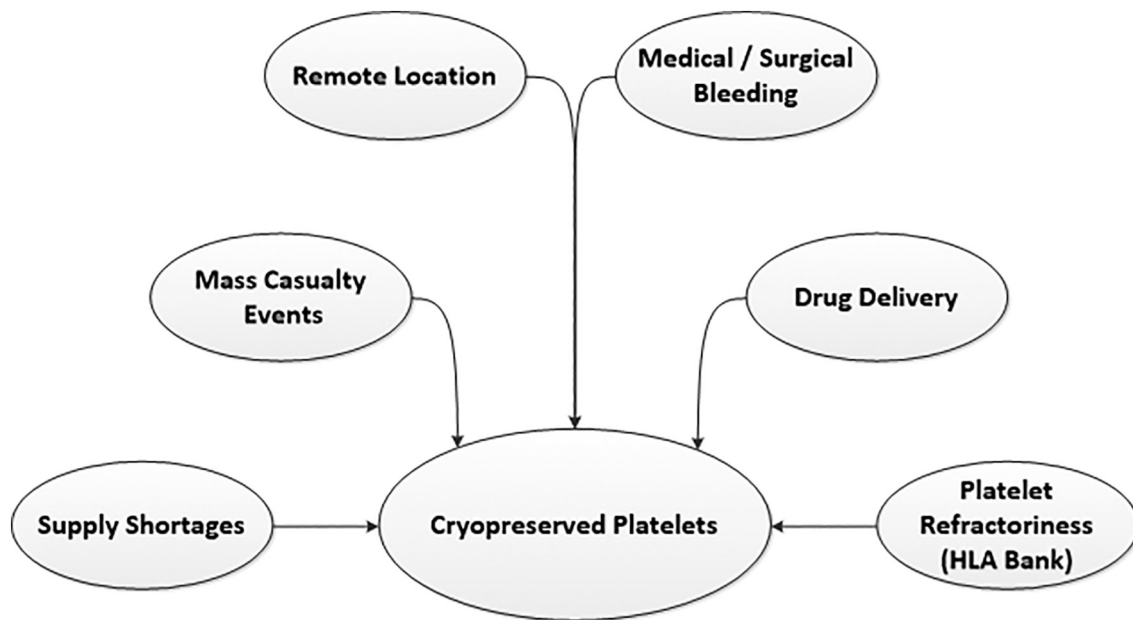


Fig. 3. Clinical applications of cryopreserved platelets.

bleeding scores, including 43% of patients with intracranial bleeding. The CPP was well tolerated and no serious adverse events were observed. This study paves the way for future studies looking into utilization of CPP in routine care of bleeding patients, including bleeding patients with hypoproliferative thrombocytopenia [53,54].

Support for Alloimmunized Patients (HLA Bank/Alloimmunization and Platelet Refractoriness)

Platelets carry on their surface class I HLA molecules which can be a target in alloimmunized patients often resulting in platelet refractoriness. Identification of HLA compatible units for alloimmunized patients is predicated on patient and donor HLA typing and availability of donors (or collected units) at the time of needed transfusion. Since HLA proteins are stable post-thaw on DMSO cryopreserved platelets [55], it would be beneficial to establish banks of HLA typed CPP units. Such banks will be able to distribute platelets to alloimmunized patients faster than the current process. Furthermore, a complete inventory profile is likely not to be necessary. It could be sufficient to have an inventory of homozygous HLA platelets (eg, A2, B7; A1, B44 etc.). This approach would minimize the number of needed donors to establish an inventory covering a significant HLA diversity among patients. The cost-benefit trade-off of such an available inventory for rapid platelet support to bleeding alloimmunized patients will need to be considered.

Drug Delivery Using Platelets

The first use of medication loaded platelets was described in 1978 as a treatment option for refractory chronic idiopathic thrombocytopenic purpura (ITP) [56]. In this study, normal platelets were incubated with vinca alkaloids and reinfused, without a significant washing step, to the patients. The results were encouraging as 6 of 11 patients had a complete remission. Similarly positive results were noted in several case reports [57]. However, when larger studies were performed the efficacy of this approach seemed to be equivalent to vinca infusion alone and within 5 years this therapeutic approach had been questioned [58]. The same approach was also used in 4 patients with autoimmune hemolytic anemia (AIHA) where 3 of them showed promising clinical responses [59]. More recently, improvements have been made in the manufacturing process with high response rates reported in patients with ITP, AIHA and Evans syndrome [60]. Preclinical studies have been

reported for platelets loaded with such proteins as ADAMTS-13 and thrombin which cannot be directly infused due to their immediate effect on a patient's coagulation system [61,62]. While platelet delivery techniques are only beginning to be developed, these initial approaches could be further extended to CPP. Here platelets could be loaded with a drug thereby providing a stable product with extended shelf-life. Additional manipulations of such platelets prior to cryopreservation may further increase specificity of their delivery. Recently, there has been interest in using platelets as a vehicle to transport compounds across the blood-brain barrier, significantly changing our ability to deliver drugs to the central nervous system [63].

Conclusion

The need for alternatives to RT stored liquid platelets has been a rich area of research for over half a century. While in the clinical setting transfusion of frozen platelets has been safe, studies with frozen platelets currently comprise a small number of study subjects. To achieve these goals, CPP manufacturing has been optimized 1) to include closed-system processing to reduce contamination risk, 2) with establishment of a potency standard that allows for increased treatment control and 3) with continued research in PR technology for platelets prior to freezing to enhance the shelf life. Unfortunately, our progress in understanding the efficacy of frozen platelets in bleeding therapy has been slowed down by lack of corporate investment. Frozen platelets remain a promising approach for the development of life-saving alternatives as well the ability to stockpile products in underserved regions and for alloimmunized patients.

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Declaration of Competing Interest

Authors declare no conflicts of interest.

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