The ice recrystallization inhibitor polyvinyl alcohol does not improve platelet cryopreservation

Cryopreservation of biologic material for long-term storage has been investigated for decades. In the field of transfusion, cryopreservation of red blood cells (RBCs) and platelets (PLTs) is of particular interest because it may overcome problems of stock management. This is an increasing concern worldwide because of a simultaneous increasing demand for (tailored) transfusion products during declining donor recruitment successes. Cryopreservation is already used in rural regions or during military operations where logistics hamper efficient stock management of transfusion products. Although multiple cryoprotectants are commercially available including dimethyl sulfoxide (DMSO), glycerol, and trehalose, none of these molecules is perfect and each has its own limitations. Deller and colleagues investigated the use of alternative molecules as a supplement or replacement for the traditional cryoprotectants. Polyvinyl alcohol (PVA) is considered a biomimetic of naturally occurring antifreeze glycoproteins, and the effect of PVA as a cryoprotectant has been mainly investigated on RBCs.[1] The cell recovery of human and sheep RBCs after cryopreservation in hydroxyethyl starch (HES) supplemented with PVA was significantly higher compared to HES alone.[2,3] Deller and colleagues extended these findings to other cell types demonstrating that cell viability of immortalized cells and metabolic activity of rat primary hepatocytes were significantly increased when cryopreservation was with DMSO supplemented with PVA compared to DMSO alone.[4] These findings led to the question if PVA could also be used as a protective supplement for cryopreservation of PLT concentrates (PCs). In this study, PCs were cryopreserved in the presence of either 6% (vol/vol) DMSO alone or DMSO supplemented with 0.5 mg/mL PVA (9 kDa; Sigma-Aldrich; n = 3). Cryopreservation and thawing protocols were as described before.[5] In brief, a 27% (vol/vol) DMSO solution (in 0.9% [wt/vol] NaCl) was added to the PC reaching a final concentration of 6% at which point the content was split in two paired samples. One of the bags was supplemented with 0.5 mg/mL PVA while the other was untreated. Both PCs were hyperconcentrated by centrifugation and resuspended in the remaining supernatant for storage at −80°C for at least 24 hours. PCs were thawed at 37°C for 8 minutes and were resuspended in 125 mL ABO-matched plasma, prewarmed to 30°C. PLT concentration and mean PLT volume (MPV) were determined with an automated hematology analyzer (PocH-1000i, Sysmex). PLT characteristics were determined by flow cytometry measuring GPIbα expression, P-selectin expression as a marker of degranulation, phosphatidylserine externalization, and microparticle formation. PLT functionality was measured by PAC-1 binding to integrin $\alpha_{IIb}\beta_3$ in response to activation with 32.5 μmol/L thrombin-related activating protein (TRAP6) and by agglutination with 1.75 mg/mL ristocetin, as described previously.[5] Fig. 1 shows that the recovery of PLTs in the presence of PVA was 73.9% (±7.9) which was not significantly different from the control with 70.4% (±10.5) recovery (p = 0.61). Cryopreservation resulted in PLT degranulation (Fig. 2A), phosphatidylserine flip to the outer leaflet of the membrane (Fig. 2B), microparticle formation (Figs. 2C and S1A), and GPIbα shedding (Figs. 2D and S1B). The impact was similar on PCs cryopreserved in the absence or presence of PVA. A slight but significantly lower expression of CD62P was found if PCs were cryopreserved in the presence of PVA compared to no PVA (p = 0.01) but the effect size was too small to be biologically relevant. PLT function was affected after cryopreservation with decreased PAC-1 binding in response to TRAP6 from 89% (±5%) before cryopreservation to 32% (±1) without and 29% (±4) with PVA after cryopreservation (Figs. 2E and S1C). This impaired functionality is confirmed by decreased PLT agglutination with ristocetin and was again not significantly different with or without PVA (Fig. 2F). MPV after cryopreservation without or with PVA was 12.9 (±0.2) and 13.0 (±0.2) fL, respectively, and was not significantly different (p = 0.69). The MPV was higher after cryopreservation compared to before (9.3 ± 0.4 fL; Fig. 2G). Cryoprotection happens by replacement of water inside the cells using an organic solvent that promotes vitrification thereby avoiding mechanical damage caused by ice crystal formation during freezing. However, ice recrystallization during thawing is another cyclic process that leads to significant cell damage and thus is a valid target for preventing cryodamage. PVA adsorbs to ice surfaces and inhibits ice crystal agglomeration in a process called ice recrystallization inhibition (IRI). PVA can enhance cell recovery and functionality

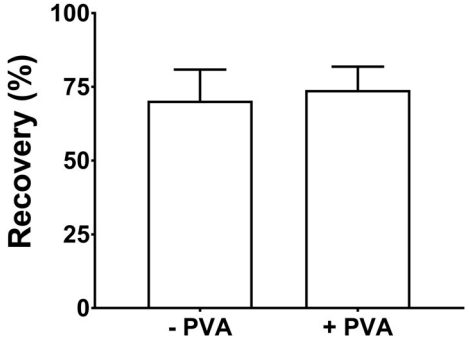

Fig. 1. PLT recovery after cryopreservation. PLT recovery (%) was the ratio of total PLT number after cryopreservation to before. Cryopreservation was in the presence of DMSO either with 0.5 mg/mL PVA (+ PVA) or without (− PVA). The number of PLTs was determined using an automated hematology analyzer. No significant difference was found by a paired t-test using computer software (Prism, Version 7.04, GraphPad Software Inc.).

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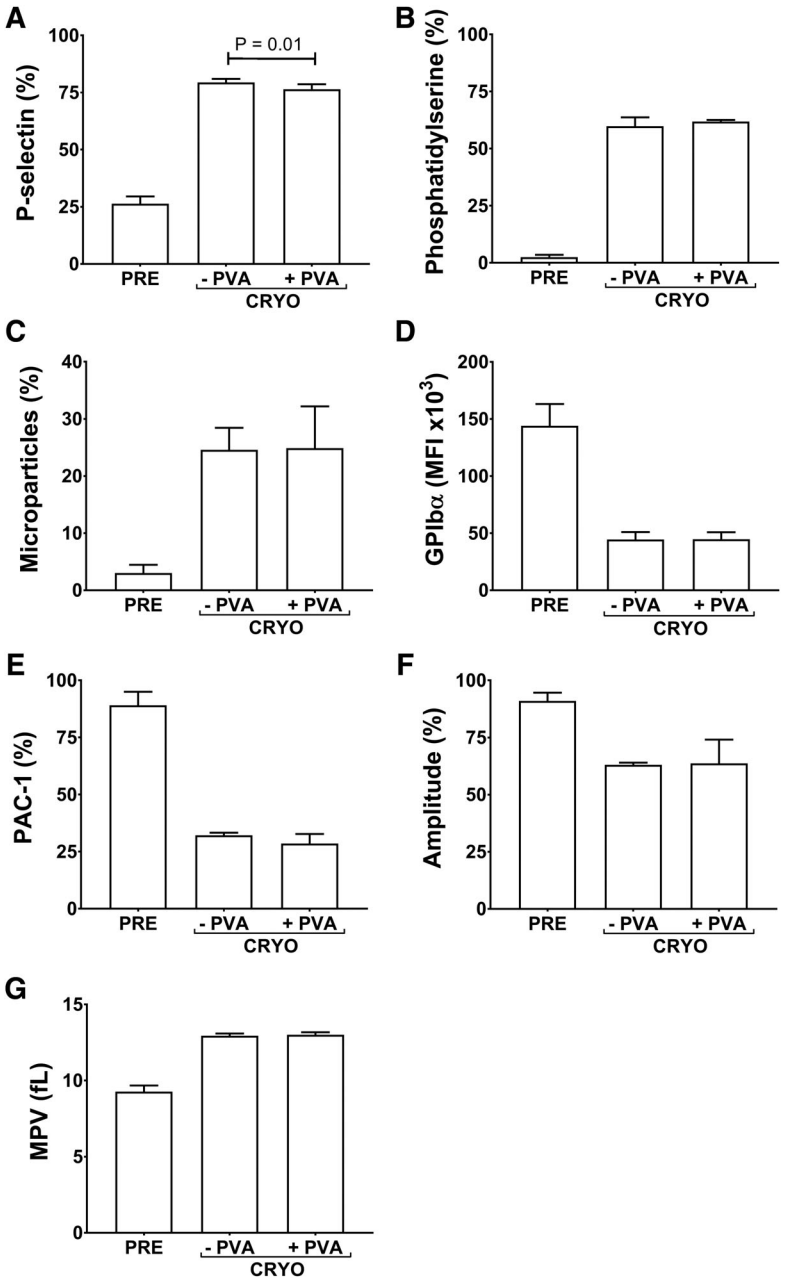

Fig. 2. PLT storage lesion after cryopreservation. Markers of PLT storage lesion were determined after cryopreservation of PC in the presence of DMSO without PVA (− PVA) or supplemented with 0.5 mg/mL PVA (+ PVA). Levels before cryopreservation (PRE) were determined as reference values. (A & B) P-selectin and phosphatidylserine expression measured by flow cytometry as markers of PLT degranulation and membrane phospholipid translocation. (C) Microparticles were defined as events smaller than 0.9 μm (with a detection limit of 0.5 μm) and positive for GPIbα. The relative number of microparticles is depicted to the total number of GPIbα-positive events. (D) Mean fluorescence intensity (MFI) as a surrogate for GPIbα expression as determined by flow cytometry. (E) The percentage of PLTs expressing integrin $\alpha_{IIb}\beta_3$ in response to stimulation with TRAP6 measured by binding of PAC-1. (F) PLT agglutination in response to ristocetin. Maximal amplitude (%) is shown. (G) MPV was determined using an automated hematology analyzer. Cryopreserved samples without or with PVA were compared for statistical differences using paired t tests (GraphPad Software Inc.).

after cryopreservation by its IRI properties and this was successfully shown for RBCs, immortalized cells, and rat hepatocytes, although the optimal PVA concentration differed between cell types. Our data now show that supplementation with PVA during cryopreservation of PLTs does not result in increased recovery nor increased PLT functionality. The results shown in this letter were obtained by addition of 0.5 mg/mL PVA but higher concentrations of the molecule were also tested without

success (data not shown). One possible explanation is that the detected cell damage is not the result of ice recrystallization and thus cannot be inhibited by addition of PVA. The mechanism leading to preactivated, damaged PLTs during cryopreservation needs further investigation and may lead to the discovery of other alternative cryoprotectants to supplement or replace DMSO, but although PVA had no cryoprotective impact on PLTs, it can enhance cryopreservation of other cell types where cell damage is caused by ice recrystallization.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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REFERENCES

1. Inada T, Lu SS. Inhibition of recrystallization of ice grains by adsorption of poly(vinyl alcohol) onto ice surfaces. Cryst Growth Des 2003;3:747-52.
2. Deller RC, Vatish M, Mitchell DA, et al. Glycerol-free cryopreservation of red blood cells enabled by ice-recrystallization-inhibiting polymers. ACS Biomater Sci Eng 2015;1: 789-94.
3. Deller RC, Vatish M, Mitchell DA, et al. Synthetic polymers enable non-vitreous cellular cryopreservation by reducing ice crystal growth during thawing. Nat Commun 2014;5:3244.
4. Deller RC, Pessin JE, Vatish M, et al. Enhanced non-vitreous cryopreservation of immortalized and primary cells by ice-growth inhibiting polymers. Biomater Sci 2016;4: 1079-84.
5. Six KR, Delabie W, Devreese KMJ, et al. Comparison between manufacturing sites shows differential adhesion, activation, and GPIbalpha expression of cryopreserved platelets. Transfusion 2018;58:2645-56.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Fig. S1 Density plots of microparticles and histograms of PAC-1 binding and GPIbα expressing events in flow cytometry (A) Representative density plot of GPIbα positive events using forward (FSC) by side scatter (SSC) analysis. Events falling in the red gate are microparticles defined as events smaller than 0.9 μm. (B) Representative histograms of GPIbα expression on platelets. (C) Representative histograms of PAC-1 binding to integrin $\alpha_{IIb}\beta_3$ on platelets activated with TRAP6. The red dotted line indicates a threshold gate for integrin activation. In all panels, graphs are for platelets before cryopreservation (left), cryopreserved without PVA (middle) or with (right) PVA.

Sero-positive blood donors to *Trypanosoma cruzi*: focus on the diagnostic

We read with great interest and in detail the study by González-Guzmán et al.[1] The authors, by employing an extensive serologic screening over 6.5 years, investigated serum samples of 510,047 blood donors and reported the prevalence of *Trypanosoma cruzi* infection in an endemic area for Chagas disease. The serologic findings are relevant and support the emerging concern that the spread of Chagas disease in endemic and nonendemic countries is also associated with poor parasitological screening of blood donors.[2] Taking into account the valuable data reported by González-Guzmán et al.,[1] we would like to comment on the study findings in perspective.

In the serologic screening, the high number of positive (n = 2613) and inconclusive samples (n = 2018) compared with the confirmed cases (n = 595) were of particular interest. Considering the divergent profiles of sensitivity and specificity, it may be relevant to contextualize the main currently available diagnostic methods. In conventional serologic methods, cross-reaction with *Leishmania* spp. and *Trypanosoma rangeli* antigens can result in false-positive serology due to some degree of molecular homology.[3] As these pathogens are also endemic in the same area investigated, internal and external serologic controls could be interesting in a differential diagnostic. Accordingly, in the case of discordant results, immunoblotting with *T. cruzi* antigens or polymerase chain reaction (PCR) methods are recommended to distinguish infections by different pathogens.[3,4]

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