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Optimizing Platelet Cryopreservation for the Battlefield: A Comparison of Conventional and Rapid-Deployment Formulations

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Introduction

Hemorrhage remains a leading cause of mortality in both civilian and military settings, underscoring the critical need for readily available and effective blood products. Conventional methods of platelet (PLT) cryopreservation using 6% DMSO is hampered by its need for a post-thaw wash and plasma reconstitution, rendering it impractical in forward settings. Our earlier studies demonstrated that cryopreserved PLTs are more procoagulant than fresh PLTs. In this work, we evaluated and compared conventionally cryopreserved PLTs with rapidly deployable (i.e. no-wash) cryopreserved PLT products designed to streamline transfusion in far-forward environments.

Learning Objectives

1. Describe the logistical challenges of conventional 6% DMSO platelet cryopreservation that limit its use in pre-hospital and battle-field environments.
2. Compare the post-thaw quality, hemostatic function and recovery rates of platelet cryopreserved with 6% DMSO, 3% DMSO, and trehalose.
3. Discuss how advances in cryopreservation techniques, such as rapidly deployable no-wash cryopreserved platelet products, can alleviate logistical challenges present in conventional storage methods

Methods

PLT units (n=20) were cryopreserved using a Vitrafy VCU1 Device (**Figure 1**) and stored in -80°C for ≥24 hours. Each unit was divided into three arms: (i) 6% DMSO, (ii) 3% DMSO, and (iii) trehalose (TREH). Post-thaw (PT), conventional samples were reconstituted with plasma prior to analysis, while 3% DMSO and TREH groups were analyzed as-is. Results were compared to a Pre-Freeze baseline. PLT quality was evaluated using complete blood count, blood gas analysis, and swirl assessment. Hemostatic function was measured using thromboelastography and thrombin generation assay. Platelet phenotype, activation status, and microparticle expression were determined using flow cytometry.

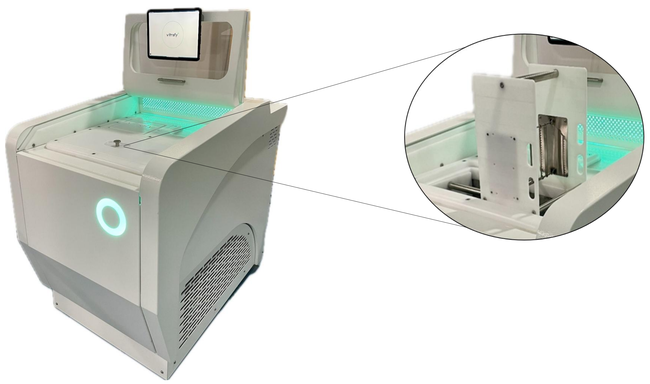
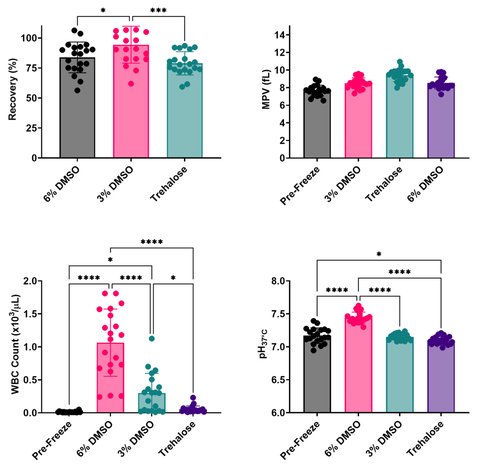


Figure 1. Vitrafy VCU1 Device

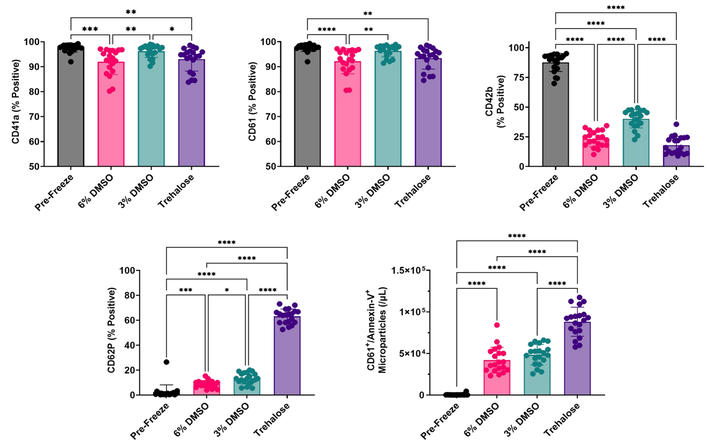
Results

PLT recovery was highest with 3% DMSO and a significant increase in WBC counts compared to pre-freeze was observed for 6% DMSO and 3% DMSO samples.



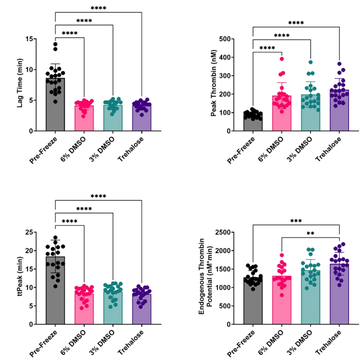
Platelet quality. Platelet quality was assessed via complete blood counts using a Beckman Coulter DxH 520 (for reporting of Platelet Recovery, mean platelet value (MPV), White Blood Cell (WBC) counts) and via i-STAT 1 metabolic analyzer (pH). Data are visualized with mean±SD for n=20 donors. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. DMSO, dimethyl sulfoxide.

3% DMSO samples led to the highest retention of glycoproteins among PT samples. Trehalose had the highest levels of activation and increased presence of platelet microparticles across all samples.



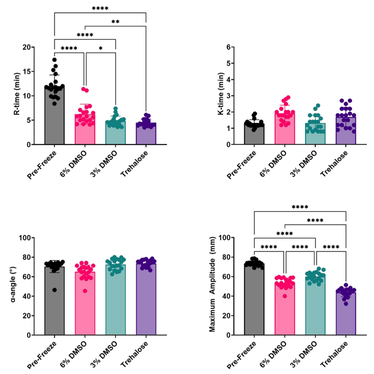
Platelet phenotype and microparticle expression. Platelet samples were stained with various antibodies and evaluated for expression of constitutive and activation markers using flow cytometry. Percent positive (%) expression of CD41, CD61, CD42b, and CD62P were reported. Absolute microparticle count was established with TruCount tubes and based on CD61 and Annexin-V positivity for events ≤1.0 µm. Data are visualized with mean±SD for n=20 donors. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. DMSO, dimethyl sulfoxide; SD, standard deviation.

Clotting time and thrombin generation were significantly faster in all PT groups compared to Pre-Freeze.



Thrombin generation. Thrombin generation was assessed using a Stago CAT analyzer. Lag time, peak thrombin generation, time to peak, and endogenous thrombin potential were recorded. Data are visualized with mean±SD for n=20 donors. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. CAT, Calibrated Automated Thrombogram; DMSO, dimethyl sulfoxide; SD, standard deviation.

Clot strength was better preserved with 3% DMSO compared to 6% DMSO and TREH, though all PT groups showed reduced clot strength vs Pre-Freeze.



Hemostatic function. Platelet samples were assessed for clotting function using a Haemonetics TEG 5000 analyzer. Clot formation time (R-time), clot amplification (K-time), clot propagation (α-angle), and clot strength (MA) were recorded. Data are visualized with mean±SD for n=20 donors. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. DMSO, dimethyl sulfoxide; MA, maximum amplitude; SD, standard deviation; TEG, thromboelastography.

Conclusions

Both conventional and rapidly deployable cryopreserved PLTs are viable and demonstrate a procoagulant phenotype. The 3% DMSO and TREH products offer a significant logistical advantage by eliminating the need for plasma reconstitution, making them better suited for pre-hospital and battlefield settings. These findings support the continued development of rapidly deployable cryopreserved PLTs as a life-saving intervention in trauma and critical care.

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