



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 401. BLOOD TRANSFUSION

**Apoptotic Pathway Inhibition in Cold-Stored Platelet Concentrates Facilitates Retained Ability to Form Thrombi**

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**Introduction:** Platelet concentrates (PCs) are used to treat or prevent bleeding in patients with impaired platelet function or after injury. However, the standard storage of PCs at room-temperature (RT) increases the risk of bacterial contamination and is associated with reduced platelet functionality due to storage lesions. Our group reported that cold-stored PCs have better functionality compared to RT-stored PCs but reduced *in vivo* circulation time due to cold-induced apoptosis. In order to investigate cold-stored PCs hemostatic functionality with and without apoptosis inhibition, we established an *ex vivo* model simulating physiological conditions of blood flow to evaluate the contribution of cold-stored PCs in thrombus formation.

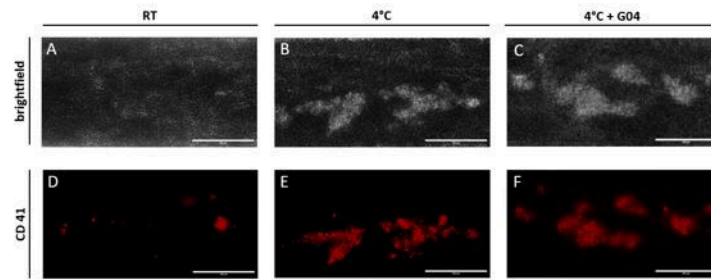
**Methods:** Apheresis-derived PCs were stored for 1, 4, 7 and 10 days under agitation at 4°C or RT either with or without the apoptosis inhibitor G04 (RhoA GTPase inhibitor). Next, PCs were stained with CD41 antibody, recalcified and incubated with TRAP6 (thrombin receptor-activating peptide 6) to initiate thrombus formation. Platelet-depleted whole blood samples from healthy donors were spiked-in with PCs and administered to the microfluidic channels of the *ex vivo* system (Bioflux), under physiological shear pressure. After five minutes of perfusion the resulting thrombi were imaged and analyzed. Ability to form thrombi was further assessed by thromboelastography and impedance aggregometry.

**Results:** Platelets from 24h storage PCs showed stable thrombus formation upon activation with TRAP6. Unstimulated platelets did not form any thrombi during the entire period of perfusion. To evaluate the effect of cold storage on platelet functions we analyzed the extent of thrombus formation of PCs stored at RT and 4°C upon CD41 staining. Our results indicate that cold-stored platelets form larger clots under flow conditions and maintain higher functionality compared to PCs stored at RT. Moreover, incubation of cold-stored PCs with the apoptosis inhibitor G04 improved *ex vivo* thrombus formation compared to PCs stored without the inhibitor, without inducing any adverse effects (Figure 1).

**Conclusion:** The results indicate that our *ex vivo* assay, which simulates PC transfusion in thrombocytopenic patients, is suitable to test the hemostatic functions of PCs under physiological flow conditions. We demonstrated that PCs stored at 4°C show better thrombus formation ability upon stimulation by agonists compared to RT-stored PCs and that the ability to form clots under shear stress is still preserved after incubation with the apoptosis inhibitor.

To conclude, cold-storage of PCs in combination with apoptosis inhibition is a promising strategy to reduce the risk of bacterial contamination and prolong *in vivo* survival time while preserving platelet's hemostatic functionality.

**Disclosures** No relevant conflicts of interest to declare.



**Figure 1: Ex vivo thrombus formation of RT and 4°C (with apoptosis inhibitor G04).**

Representative brightfield (upper panel) and immunofluorescence (lower panel) pictures of *ex vivo* thrombi formed on collagen-coated channels. Spike-in-PCs, stored at RT (A and D), 4°C (B and E) or 4°C + G04 (C and F) were perfused through microfluidic channels, upon TRAP6 stimulation, for 5 minutes. Prior to perfusion samples were stained with CD41 antibody. Red signal: CD41 antibody. Scale bar: 200  $\mu$ m.

**Figure 1**

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