Zeodration: a new method for the preservation of blood platelets

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Objectives
Blood platelets for transfusion have to be stored in restricted conditions (5 days, 22°C, under stirring). So far, procedures for long-term storage are unsuccessful due to overwhelming drawbacks (cold exposure, non insufible protective agent).

We adapted zeodration to platelets, a dehydration process consisting of water evaporation under vacuum. This method can be performed in a large range of temperatures, notably at room temperature (RT).

1. Zeodration process

Zeodration process is performed at room temperature, in a confined atmosphere, under vacuum (145 mbar) and provided a highly dry state final product. Zeodrated platelet (Z_plt) storage is conducted at RT, under low residual air and protected from UVs.

Methods

Washed platelets were reexpended in Tyrode's buffer containing 0.35% or 5% (w/v) human serum albumin (HSA) and maintained at 37°C before zeodration.

Platelet count, platelet distribution width (PDW), and mean platelet volume (MPV) were measured with a Sysmex® XE-2100 automated hematology system using a sheath flow DC detection method.

Platelet morphology: fixed fresh and zeodrated platelets were observed by scanning and transmission electron microscopy.

Flow cytometry (FCM) was performed using the Becton Coulter Galios®.

Platelet agglutination and aggregation were measured in a Caen TXA4 aggregometry (Evtec GmbH).

Platelet adhesion under flow: Fresh (F_plt) and zeodrated platelet (Z_plt) suspension were mixed with heparinized red cell concentrates (R5C) and perfused into a poly(dimethylsiloxane) (PDMS) flow chamber coated with vWF on collagen. The platelet behavior was visualized and analyzed with the MetaMorph and Image J softwares 3D reconstructions were performed with Anima software.

Procoagulant activity: Annexin-V binding (FCM) and Calibrated Activated Thrombin (CAT) generation experiments were performed using the thrombinogram method in a fluorescence plate reader (Fluorometer Assay®: Thrombinlab®) detecting cleaved fluorescent thrombin substrate Z.GGP-FAM accumulation in reconstituted (PPF, F_plt or Z_plt, PPF, vv).

Rehydration method was optimized with a first step atmosphere condensation in environmental chamber (SECASI Technologies), following dilutional water addition.

Abbreviations: RT - room temperature; Uva - ultra-violet; HSA - human serum albumin; PDW - platelet distribution width; MPV - mean platelet volume; FCM - flow cytometry; F_plt - fresh platelet; Z_plt - zeodrated platelet; CAT - calibrated activated thrombin; HAB - humanized antibody; PPF - citrated platelet-rich plasma; PPDF - citrated platelet poor plasma; vWF - von Willebrand Factor; Fb - Fibrogen; PDMS - poly(dimethylsiloxane); HFA - Human Fibrinogen, Kabila.


Conclusions
Platelets can be dried by the zeodration process2. Z_plt can be stored for a long period of time without using stabilizing agent. Z_plt exhibit an activated pattern, a preserved agglutination and adhesion to vWF and to collagen under flow.

Whether Z_plt preserve hemostatic properties will be studied by transfusion in animal models.