Zeodration : a new method for the preservation of blood platelets

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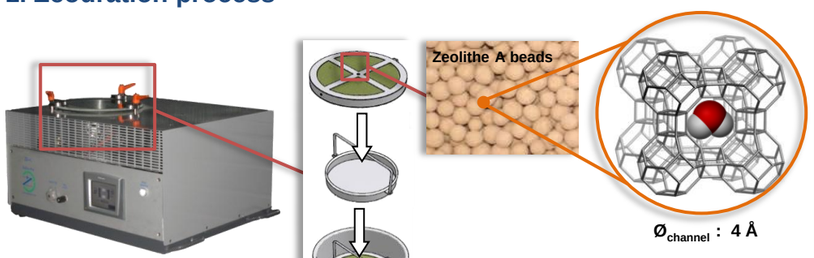
Objectives

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 infusible 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



Lab scale zeodrator (ZDL+)

Process parameters

Zeodration time	2 h 34 ± 16 min
Average temperature	20.2 ± 1.4 °C
Final relative humidity	0.60 ± 0.2 %
Final water activity	0.301 ± 0.01

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[1] were resuspended 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[2].

Flow cytometry (FCM) was performed using the Beckman Coulter Gallios®.

Platelet agglutination and aggregation were measured in a Carat TX4® aggregometer (Entec GmbH)

Platelet adhesion under flow: Fresh (F_plt) and zeodrated platelet (Z_plt) suspension were mixed with hirudinated red cell concentrates (v/v) and perfused into a polydimethylsyloxane (PDMS) flow chamber coated with vWF or collagen. The platelet behavior was vizualized and analyzed with the Metamorph and Image J softwares 3D reconstructions were performed with Amira software.

Procoagulant activity: Annexin-V binding (FCM) and Calibrated Automated Thrombin (CAT) generation experiments were performed using the thrombogram method in a fluorescence plate reader (Fluoroskan Ascent ®; ThermoLabsystems) detecting cleaved fluorogenic thrombin substrate Z-GGR-AMC accumulation in reconstituted cPRP (F_plt or Z_plt : cPPP, v/v).

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

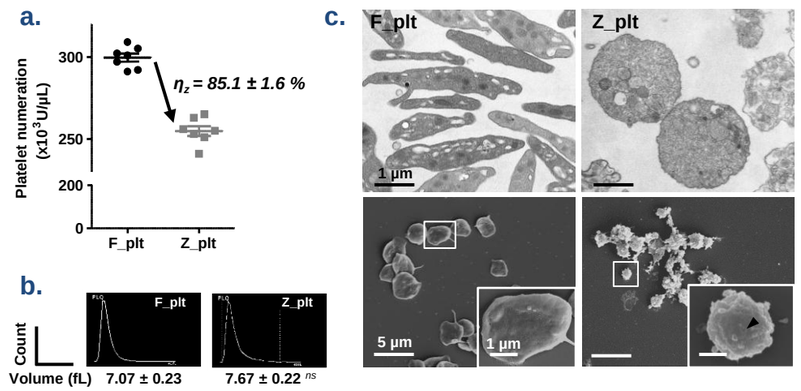
***Abbreviations**: RT : room temperature; Uvs : ultra-violets; HSA : human serum albumin; PDW : platelet distribution width; MPV: mean platelet volume, FCM : flow cytometry; F_plt : fresh platelet; Z_plt : zeodrated platelet; CAT : calibrated automated thrombin; hAb : humanized antibody; cPRP : citrated platelet rich plasma; cPPP : citrated platelet poor plasma; vWF : von Willebrand Factor; Fb : fibrinogen; PDMS : polydimethylsiloxane; HFK : Human Fibrinogen Kabi.*

[1] Cazenave JP *et al.*, Preparation of washed platelet suspensions from human and rodent blood, **Method Mol Biol**, *2004*

[2] Eckly A *et al.*, Characterization of megakaryocyte development in the native bone marrow environment, **Method Mol Biol**, *2004*

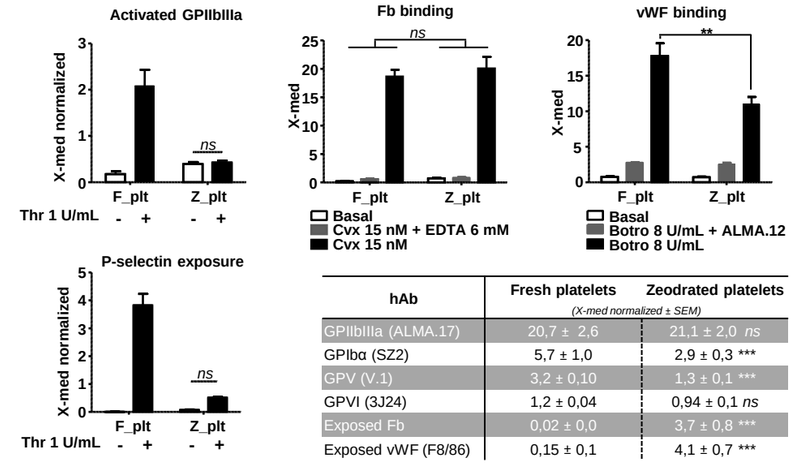
[3] Rendu F, Donnet T, Gachet C, Cazenave JP, Zeodration method for the preservation of blood platelets, **WO/2011/124280**

2. Z_plt recovery rate and morphology



a. The amount of Z_plt recovered after rehydration is closed to the initial count. Recovery rate is improved by using **PDMS coating supports** and **addition of 5% HSA** (w/v). *b.* PDW measure shows a well-preserved profile. *c.* Z_plt has a **round** morphology and a **condensed** cytoplasm compared to fresh platelets (F_plt). The **granules content** is partly conserved whereas **membrane continuity** is lost (arrow head).

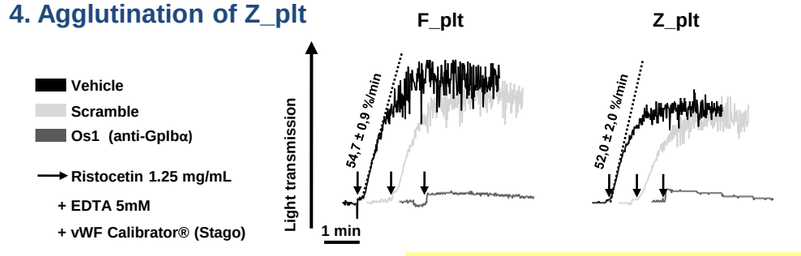
3. Z_plt activation markers and glycoprotein expressions



hAb	Fresh platelets	Zeodrated platelets
	(X-med normalized ± SEM)	
GPIIbIIIa (ALMA.17)	20,7 ± 2,6	21,1 ± 2,0 *ns*
GPIbα (SZ2)	5,7 ± 1,0	2,9 ± 0,3 ***
GPV (V.1)	3,2 ± 0,10	1,3 ± 0,1 ***
GPVI (3J24)	1,2 ± 0,04	0,94 ± 0,1 *ns*
Exposed Fb	0,02 ± 0,0	3,7 ± 0,8 ***
Exposed vWF (F8/86)	0,15 ± 0,1	4,1 ± 0,7 ***

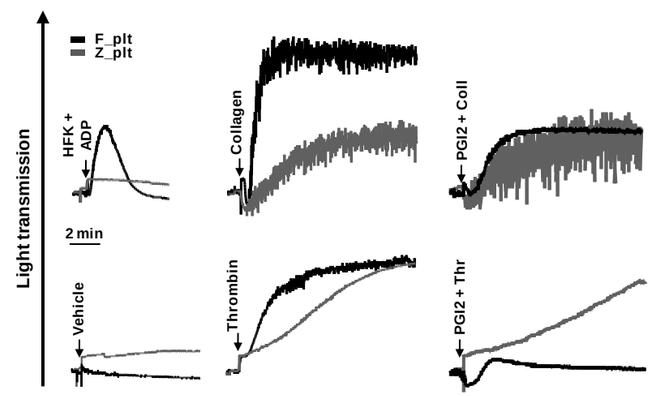
Activation markers are exposed on Z_plt surface. However, ability to **specifically bind** vWF and fibrinogen is preserved.

4. Agglutination of Z_plt



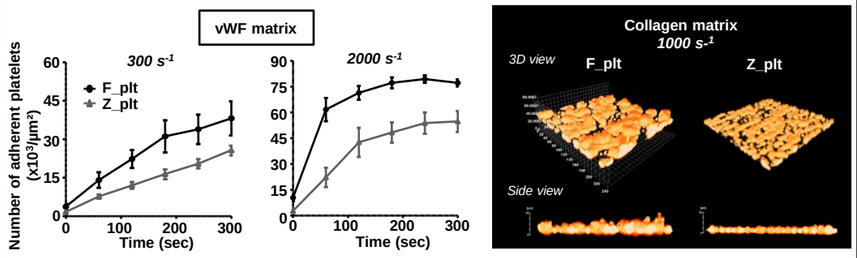
Z_plt bridging via **GpIb-vWF** is preserved.

5. Aggregation of Z_plt



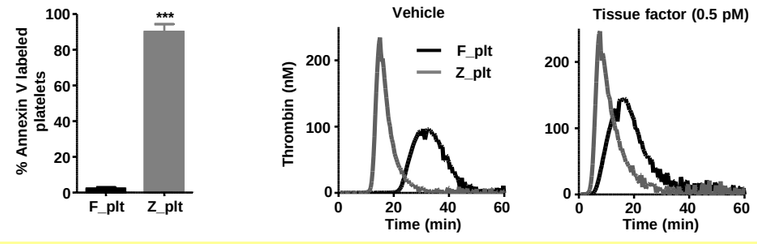
Z_plt failed to aggregate in response to ADP stimulation (5 to 100 µM). Collagen (5 to 50 µg/mL) and thrombin (0.1 to 1 U/mL) led to an aggregation signal. Responses to collagen and thrombin are **independent of platelet metabolism** as PGI2 treatment suggested.

6. Z_plt adhesion under flow conditions



Z_plt are able to **adhere** to immobilized vWF and to **form thrombi** on a collagen matrix at various **shear rates.**

7. Z_plt pro-coagulant activity



Z_plt **exposed phosphatidylserine** at their surface and promoted **thrombin generation.**

Conclusions

Platelets can be dried by the zeodration process[3]. 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 conserve hemostatic properties will be studied by transfusion in animal models