# Acoustophoretic preparation of platelet concentrate: a relevant and practical alternative to centrifugation

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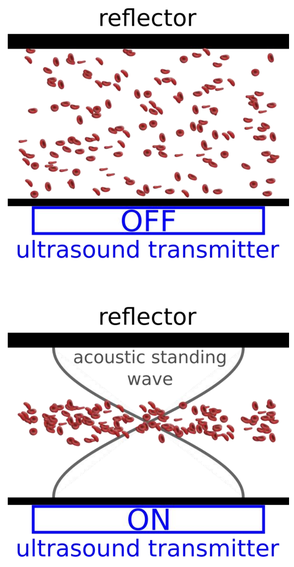
## Introduction

Shear-induced platelet activation is an unwanted side effect of the centrifugation-based procedure currently used in blood banks to prepare platelet concentrates.

Reducing platelet activation during blood blank fractionation process is of fundamental importance to improve both platelet concentrate quality and recipient safety.

The principle used with this technology is based on **acoustic levitation**. Indeed, it's possible to exert forces on micro-object in suspension, including **cell, bacteria, yeast, microalgae with an ultrasonic standing wave**. The acoustic radiation force depends on the **intrinsic properties** of the handled object : **size, density and compressibility**.

In whole blood, platelets and red blood cells have not the same acoustic properties, so the **acoustic radiation force is 4 times greater on red blood cells than platelets**. Thanks to their physical difference, we are able to separate red blood cell to platelet with **non-contact, gentle and continue process**.




In this study we describe an innovative acoustophoretic device dedicated to whole blood fractionation. Our objectives were to evaluate the efficiency of the separation between red blood cells and platelets and to evaluate the impact of this new cell sorting technology on the quality and functionality of acoustically-isolated platelets.

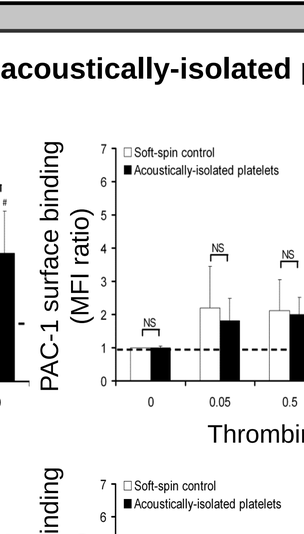
## Material and Methods

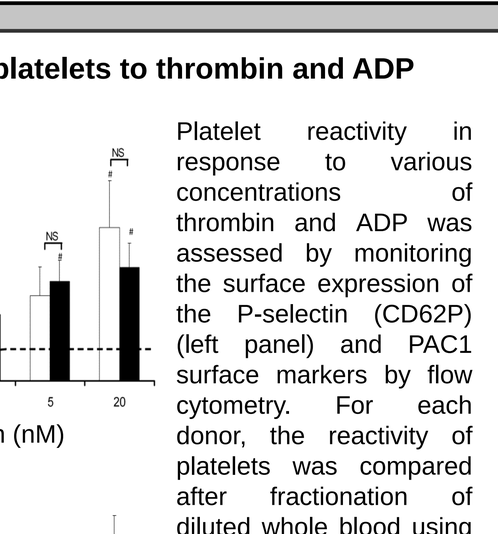
$$F_{acoustic} = 4\pi r^3 \psi(\beta, \rho) k_0 E_0 \sin(2k_0 z)$$

Intrinsic properties of the object :
- size of the objet
- acoustic properties of the object (density, compressibilité)

Experimental parameters :
- frequency
- acoustic energy

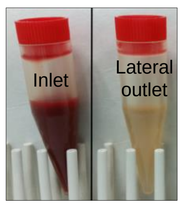
The flow of 1.5ml/min in inlet and 0.5ml/min in two lateral outlet is produced with three peristaltic pumps. The acoustic energy and the flow are finely tuned to focus the red blood cell in the central outlet without let the time to platelet to move in the centre.



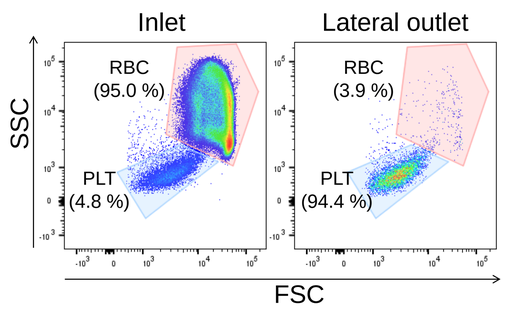




## Results

### Figure 1. Platelet separation using the acoustophoretic device




Comparison of whole blood (left) and a platelet suspension collected from the acoustic microchannel outlet (right).




Flow cytometry profiles of samples collected before and after separation using the acoustic device.

FSC - forward scatter, SSC - side scatter, PLT - platelets, RBC - red blood cells.

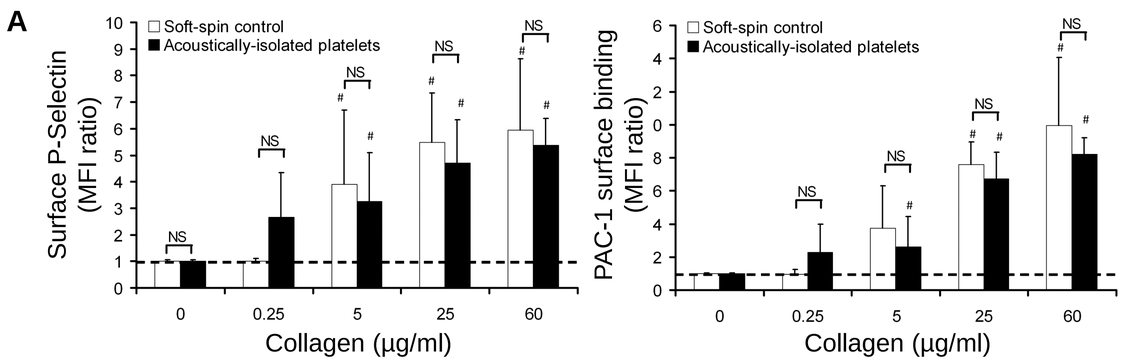
### Table 1. Blood fractionation efficiency

| Blood cell distribution and platelet indices n=14 blood donors | Diluted and leukocyte-depleted blood | Platelet suspension preparation | |
|---|---|---|---|
| | | Acoustophoretic method | Soft-spin control |
| *Platelets* | | | |
| Mean % ± SD | 6.0 ± 2.2 | 92.8 ± 12.8*** | 100.0 ± 0.0*** |
| Mean concentration ± SD (x$10^9$/l) | 55.9 ± 21.0 | 31.9 ± 14.6*** | 42.8 ± 6.6 |
| Mean yield in concentration ± SD (%) | - | 58.3 ± 19.3 | 76.0 ± 6.4 |
| *Red blood cells* | | | |
| Mean % ± SD | 94.0 ± 2.2 | 7.2 ± 12.8*** | 0.0 ± 0.0*** |
| MPV (mean ± SD) (fl) | 7.8 ± 0.6 | 6.6 ± 0.4** | 6.9 ± 0.4** |
| PDW (mean ± SD) (%) | 13.7 ± 2.0 | 9.6 ± 0.7** | 10.9 ± 1.5** |

Cell counting assessed using an automated haematology analyzer.
** P< 0.01, *** P < 0.001 *versus* unfractionated blood (non-parametric Wilcoxon signed-rank test).

Blood fractionation using the acoustophoresis technology leads to a **red blood cell clearance** ratio from whole blood **greater than 80 % (p < 0.001**) and a **purity of platelets close to 93.0 %**.

### Figure 2. Quality of acoustically-isolated platelets

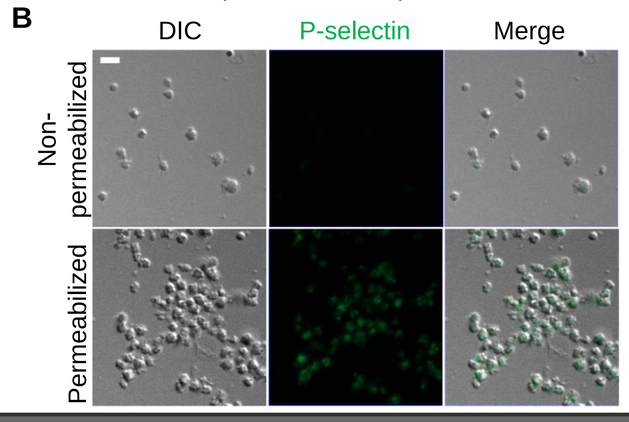



Platelet activation was evaluated by monitoring the surface expression of the fluorescent P-selectin (CD62P) (left panel) and PAC1 (right panel) by flow cytometry.

The histogram represents the ratio of mean fluorescence intensity (MFI) quantified for each activation marker before and after blood fractionation using acoustophoresis (black plots) *versus* soft-spin technique (white plots).

Post-fractionation was investigated through dose-response experiments to collagen (0.25 to 60µg/ml). Results are expressed as percent relative to the mean fluorescence intensity (MFI) of control platelets.

n = 14 different blood donors. # indicates a significant difference ($p < 0.05$) as compared to non-stimulated control platelets. PLT - platelets, MFI - mean fluorescence intensity, NS – non significant.




Representative images of acoustophoresis-isolated platelets in differential contrast (DIC) and P-selectin (CD62P). Bar = 5 µm

The **degree of platelet activation**, as attested by the surface expression of P-selectin and PAC1, **does not increase** following acoustic blood fractionation.

### Figure 2. Responsiveness of acoustically-isolated platelets to thrombin and ADP



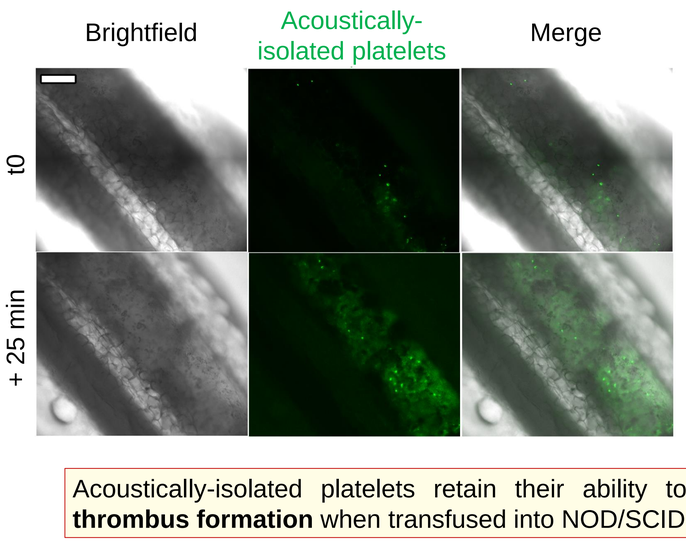

Platelet reactivity in response to various concentrations of thrombin and ADP was assessed by monitoring the surface expression of the P-selectin (CD62P) (left panel) and PAC1 surface markers by flow cytometry. For each donor, the reactivity of platelets was compared after fractionation of diluted whole blood using acoustic (black plots) and soft-spin (white plots) methodology.

(n=5 different blood donors). # indicates a significant difference ($p<0.05$) as compared to non-stimulated control platelets.

MFI : mean fluorescence intensity.

Acoustically-isolated platelets maintain their ***in vitro*** **reactivity**.

### Figure 3. *In vivo* functionnality




Acoustophoresis-isolated platelets were stained with DIOC-6 and washed *prior* to transfusion in NOD/SCID mice that were subjected to $FeCl_3$-induced thrombosis in mesenteric microvessels. Labelled platelets were injected 15 min after initiating thrombosis.

Images were taken during the first minute following their injection (t0) and 25 min later (+25 min).
Bar = 50 µm.

Acoustically-isolated platelets retain their ability to **circulate *in vivo* and contribute to thrombus formation** when transfused into NOD/SCID mice.

## Summary

**The acoustophoresis device :**

- ✓ leads to an efficient separation of platelets from whole blood;
- ✓ preserve the quality and the functionality of platelets;
- ✓ represents a novel promising technique for whole blood fractionation in clinical settings.

## Perspectives

- Quality and functionality of acoustically-isolated platelets **upon storage** (J5, J7).
- *In vivo* **recirculation assay** of acoustically-isolated platelets.
- Quality and functionality of acoustically-isolated **red blood cells** and **plasma**.
- **Improve the effiency of blood cells separation** by optimizing the resonator dimensions.
- **Increase the separation flow** in order to **decrease** the blood bag processing.
- Create a **continious process totally automated**.

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