Contactless platelets isolation using acoustic radiation forces

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Background

The isolation of platelets from platelet-rich plasma (PRP) is a critical step in various medical applications, including tissue engineering, wound healing, and regenerative medicine. The use of acoustic forces to separate platelets from PRP offers a non-invasive and efficient approach to achieving this goal. Acoustic radiation forces mobilize cells in flow according to their volume and density without contact. The centrifugation-based procedure currently employed in blood banks to create platelet concentrates leads to undesirable shear-induced activation of platelets. Mitigating this platelet activation during the blood bank's fractionation process holds paramount importance as it serves to enhance both the quality of platelet concentrates and the safety of recipients.
The technology's fundamental principle is rooted in acoustic levitation. Through the use of ultrasonic standing waves, it becomes feasible to apply forces to micro-objects suspended within the solution, encompassing cells, bacteria, yeast, etc. The magnitude of the acoustic radiation force is contingent upon the inherent traits of the handled object—namely, its dimensions, density, and compressibility.
In the realm of whole blood, platelets and red blood cells exhibit distinct acoustic characteristics, resulting in a fourfold amplification of the acoustic radiation force acting upon red blood cells as compared to platelets. This dissimilarity in physical attributes empowers the separation of red blood cells from platelets through a process that is both contactless and gentle, allowing for continuous operation.

Technology and method

10 whole blood bags were collected and processed at the French blood bank, to obtain a platelet-rich plasma (PRP) after centrifugation (1300 rpm; 13 min). The PRP bags are then immediately injected at a flow rate of 5 mL/min into the consumables in the machine prototype where they are subjected to acoustic radiation (frequency: 2.5-2.6 MHz, power of 10 and 30 W). The final volume of obtained Platelet Concentrates (PCs) was adjusted by the addition of a platelet preservative solution (Intersol, Fresenius Kabi). The 9 main standard PCs obtained after acoustophoresis were pulled, leukoreduced, and treated for pathogen attenuation by the INTERCEPT blood system. Physicochemical and biological parameters (yield, platelet content, pH, pO2, pCO2, LDH, glucose, lactate, soluble p-selectin) assays were measured at Day2, 3, 6 and 7. The samples were stored between +22°C ±2°C during the storage period.

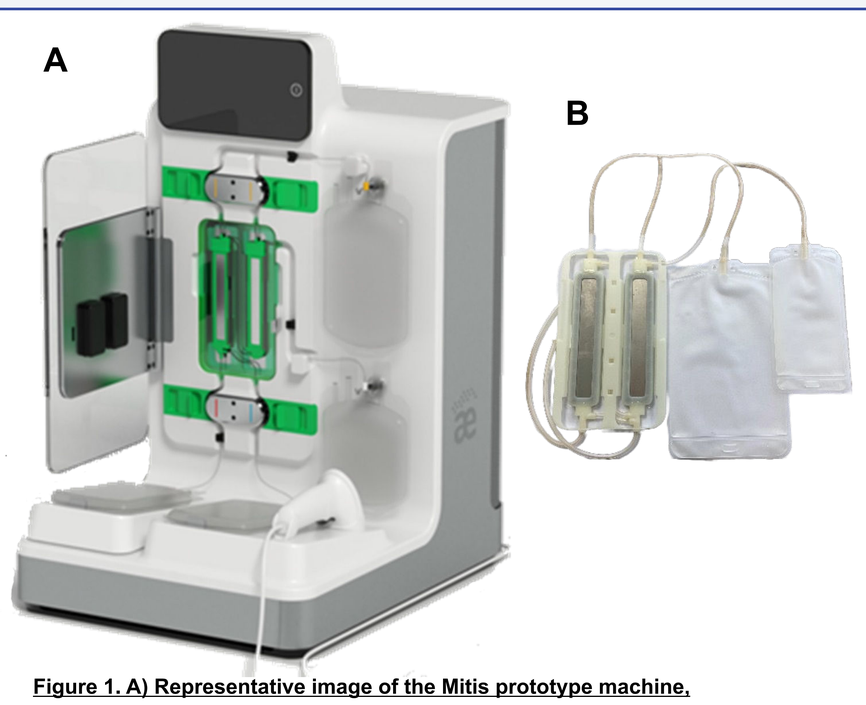

Figure 1. A) Representative image of the Mitis prototype machine, B) fully enclosed and sterile acoustic disposable

Number of sample n= 10	Volume after PRP prepartion	Platlete count 10 [11]	Platlet swirling factor	Platlet yeild PRP/whole blood
Average	344	1,5	+++	0,87
Standard deviation	16	0,2	0	0,07
Median	345	1,5	+++	0,90
Min	310	1,1	+++	0,73
Max	364	1,9	+++	0,96

Table 1. Characterization of PRP sample that has been used for this study

Metabolic characteristics of platelets

Number of samples= 10	Volume (ml)	Platlete count 10 [11]	Platlet swirling factor	Glucose (mM)	PH	Platlet yeild PRP/whole blood
Average	97	0,95	+++	9,2	7,20	0,65
Standard deviation	9	0,17	-	1,3	0,05	0,08
Median	95	0,90	+++	8,7	7,21	0,67
Min	85	0,75	+++	7,6	7,12	0,50
Max	114	1,25	+++	11,0	7,28	0,74

Table 2. Characterization of CPs after the acoustophoresis
The table shows the quantification of yield of platelet count and the vital parameters right after the acoustophoresis before storage.

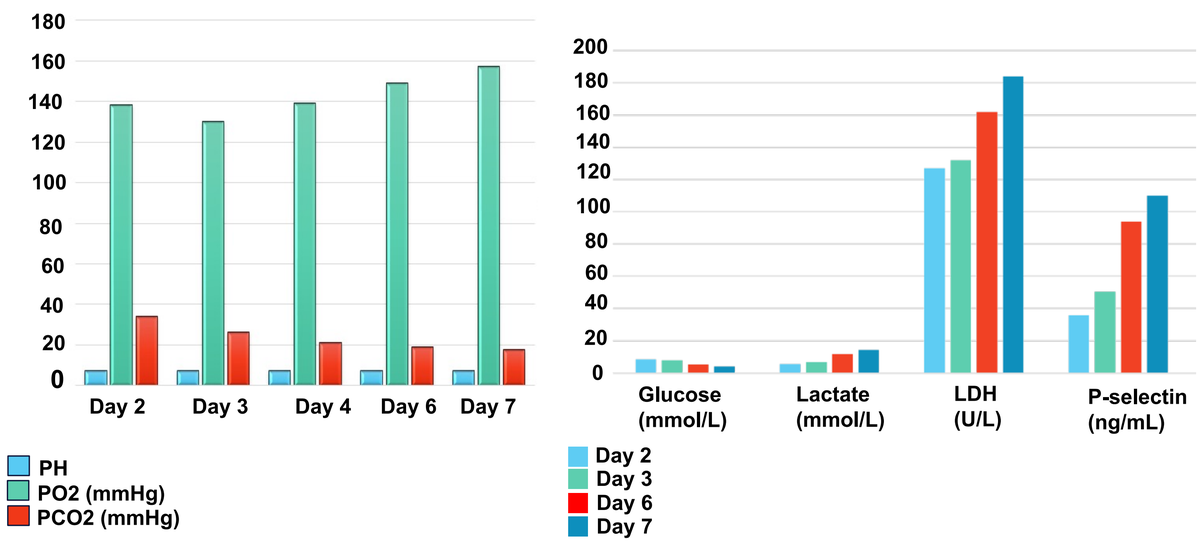

Figure 2. Quantification of platelets concentrate after acoustophoresis on pulled PCs The table shows the quantification of the vital parameters 2, 3,4,5, 6 and 7 in storage after the acoustophoresis (samples were stores at +22°C ±2°C)

Results: The result of the main factors of platelet functionality showed a good preservation of the gases (PO_2 and PCO_2) and the PH. A slight decrease of the PH was observed after the storage at day 7. The evolution of the partial pressure of oxygen (pO2) increases moderately during the conservation of the 2 Mixed Concentrated Platelet (MCP), while the pCO2 decreases during the same period. Glucose concentration decreases during the storage period, reflecting consumption of the residual plasma glucose contained in the various CPs. Glucose still remains in the 2 MCP at day 7 and day 8.
Lactate production evolved in a complementary manner to glucose consumption in the 2 MCP. The measurement of soluble P-selectin concentration shows an increase only after day 6.

Isolated plasma characteristics

Number of samples= 10	Volume (ml)	Platlete count 10 [11]	Protein content (g/L)	leukocyte residue (10^6/L)
Average	231	0,23	59,3	0,66
Standard deviation	15	0,16	2,6	0,00
Median	233	0,19	58,8	0,66
Min	310	1,1	56,2	0,66
Max	364	1,9	63,1	0,66

Table 3 characterization of the isolated plasma after the leucodepletion

Results: The 10 units of plasma obtained after separation by acoustophoresis have an average volume of 231 ± 15 ml before sampling. This average volume is lower than the average volume obtained for routine production (287 ± 20 ml in 2022).

Conclusions:

Our recent study using our prototype acoustophoresis machine to isolate platelets from Platelet-Rich Plasma (PRP) presents a promising result according to the European standard of platelets isolated from buffy coat that could lead to a transformative approach in the field of biomedical research and clinical applications in both Europe and the USA. This innovative method harnesses the power several acoustic forces to precisely and efficiently separate platelets from the complex mixture of blood components in PRP.
Acoustophoresis offers significant advantages in the context of platelet isolation by pulling the 4 CPs instead of 8. It enables rapid and selective separation, ensuring that the platelets, rich in bioactive factors, are obtained with high purity and viability. Importantly, this technology eliminates the need for harsh chemicals or traditional centrifugation methods, which can often compromise the integrity of platelets and result in suboptimal PRP quality.

Perspectives:

According to the biological parameters tested, the quality of produced PCs by acoustophoresis are comparable to the PCs under conventional production conditions. These data need to be consolidated with a larger number of procedures to be carried out. From a quantitative point of view, optimisations of the overall process will be integrated to the final devised in future developments.

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