Acoustophoretic separation:
A way to purify cultured platelets suspensions for potential clinical use?

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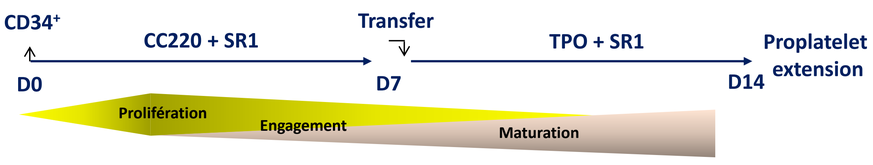
Introduction: *In vitro* production of blood platelets is currently possible. However, for cultured platelets to be considered as a real transfusion alternative, it is mandatory to properly separate them from the residual megakaryocytes and cell fragments and to demonstrate that these platelets are free of contaminants.

Our goal is to evaluate the efficiency of an acoustic-based fractionation device to separate newly released platelets (NRP) from residual megakaryocytes (MK) and cytoplasmic elements (CE).

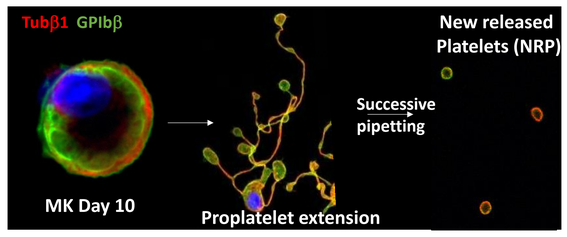
1. Platelet preparation



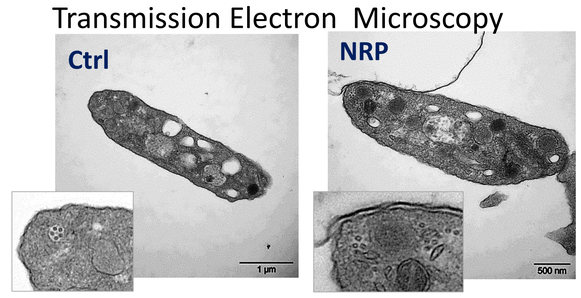
Experimental protocol : CD34+ are cultured over SR1 during 14 days (Strassel et al, Blood 2016, US2018216068).

2. Platelet morphology analysis

Immunofluorescence



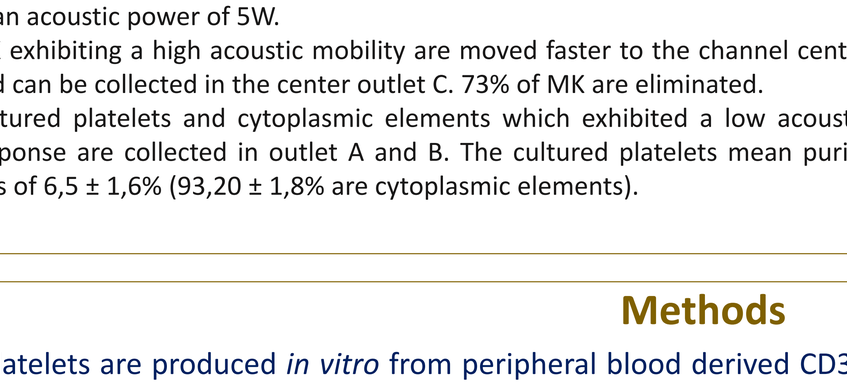
Transmission Electron Microscopy



Platelets are discoids. Their morphology is sustained by a ring of microtubules

3. Megakaryocyte elimination

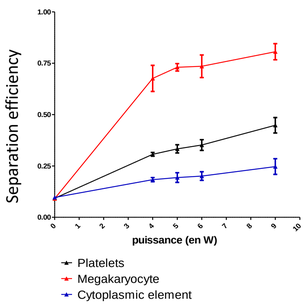
➢ Acoustofluidic channel design



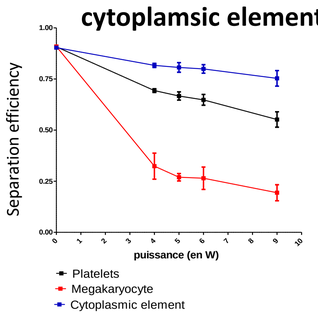
At this first step of procedure, platelet suspension is injected into the lateral inlets while StemSpan is injected in the central channel. Each inlet and outlet is driven at 0,5ml/min. An acoustic wave at 1,85MHz is applied to displace cells toward the C outlet.

➢ Platelet enrichment efficiencies

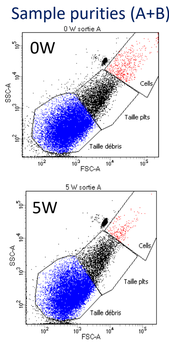
Channel C (MK elimination)



Channel A and B (cultured platelets + cytoplamsic elements)



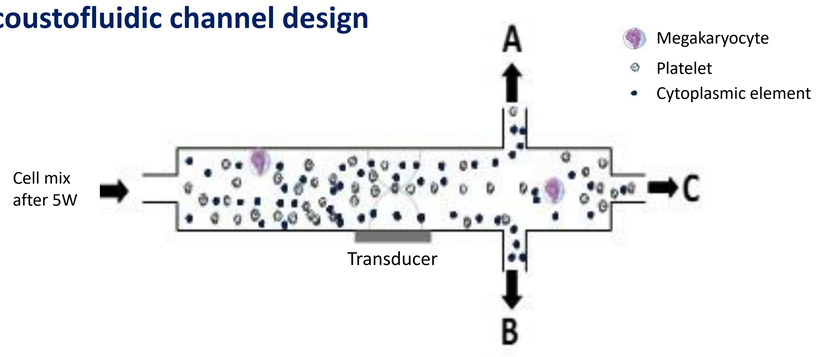
Sample purities (A+B)



The optimal platelet suspension fractionation by acoustophoresis is obtained at an acoustic power of 5W.
MK exhibiting a high acoustic mobility are moved faster to the channel center and can be collected in the center outlet C. 73% of MK are eliminated.
Cultured platelets and cytoplasmic elements which exhibited a low acoustic response are collected in outlet A and B. The cultured platelets mean purity was of 6,5 ± 1,6% (93,20 ± 1,8% are cytoplasmic elements).

4. Platelet enrichment

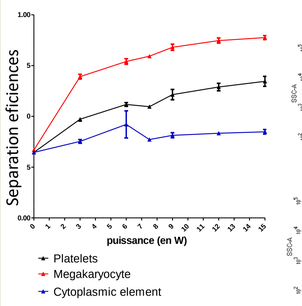
➢ Acoustofluidic channel design



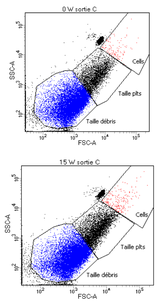
At this second step of purification, the cultured platelet suspension treated for MK elimination is injected into the focusing channel. The acoustic wave frequency and outlets flow rates are identical to the previous step. The inlet is driven at 1,5ml/min (3x0,5ml/min).

➢ Platelet enrichment efficiencies

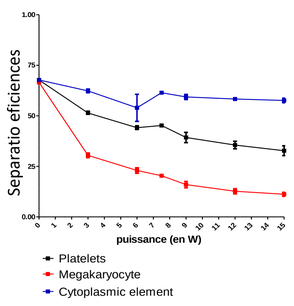
Channel C (Cultured platelet enrichment)



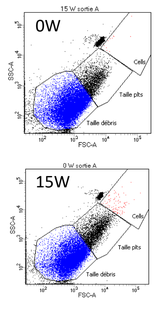
Sample purities (C)



Channel A and B (mix)



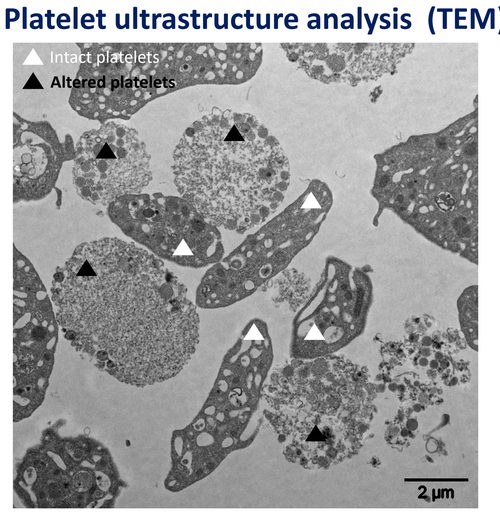
Sample purities (A+B)



The optimal cultured platelets separation is obtained at an acoustic power of 15 W.
66% of cultured platelets are collected in channel C while 58% of the cytoplasmic elements are removed. Channel A and B contained the unaffected elements (platelets, MK and cytoplasmic elements).

5. Platelet Ultrastructure and function after acoustophoresis

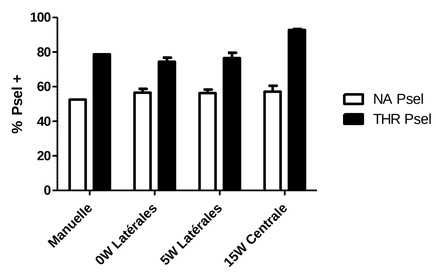
➢ Platelet ultrastructure analysis (TEM)



Most of the cultured platelets remain well organized, and maintain a typical discoid shape

➢ Platelet function analysis (Flow cytometry)

Expression of P-selectin on activated platelets with thrombin.



The activation status of acoustophoresis-isolated platelets is similar to those of platelets isolated by soft-spin centrifugation
(Cazenave et al, methods mol biol, 2004).

Methods

Platelets are produced *in vitro* from peripheral blood derived CD34 progenitors following the protocol described in Strassel et al., Blood 2016. Acoustic separation is performed according to the protocol described in Bohec et al., Platelets 2017. Platelets are released from the suspension following successive pipetting. Platelet recovery and purity after acoustic fractionation were evaluated and compared to the standard centrifugation protocol.

The acoustic-based method developed by Ænitis Technologies could be a promising alternative to purify cultured platelets in view of future clinical use. However, further improvement is required in order to increase the yield of recovery and integrity.