Low mechanical stress and high throughput concentration of cells with acoustophoresis

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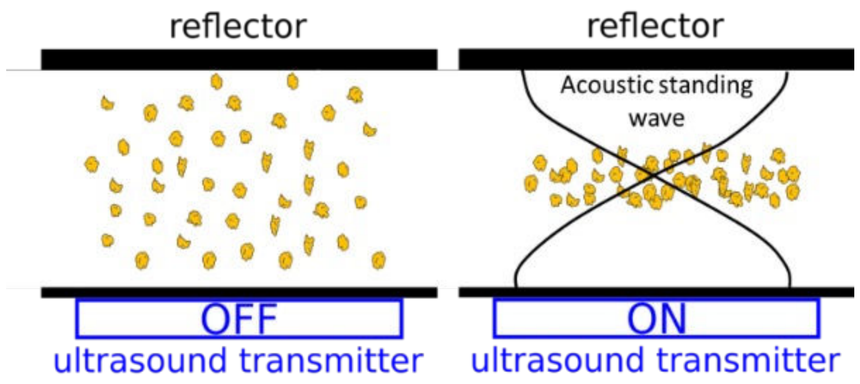
Background

Acoustophoresis is an ultrasound based technique to replace most cell sorting and washing devices such as centrifuges. It uses low acoustic radiation forces to make cells migrate to a pressure node. Unlike a centrifuge, Aenitis' technology works completely in flow and can be part of a more complex fluidic system. It can be used to sort, wash and concentrate all kinds of cells and tissues, with no change in cell viability. Flow rates can range from 0.5 ml/min to 40 ml/min. Previous results have shown that acoustophoresis can be used safely on platelets[1].

In this study, we present results obtained in a one inlet three outlets configuration, in order to concentrate cells by a factor 2.8-3.0. Multiple channels in series would increase the multiplying factor accordingly. This study shows that the reconcentration factor stayed constant for a wide range of flow rates, from 5 ml/min to 40 ml/min, with a 2.6 MHz acoustic wave. We investigated Jurkat cells and red blood cells, finding similar results.



Method

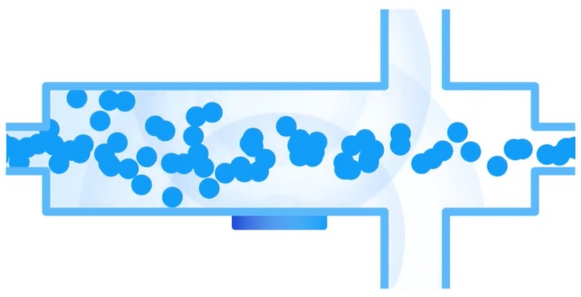


Fig.1: 1 entrance 3 outlet channel for concentration of cells

$$F_z^{rad} = 4\pi a^3 k E_{ac} \sin(2kz)\, \Phi(\rho,\kappa)$$

Acoustic radiation force: a is the cell radius, **Φ** the acoustic contrast factor, **k** = 2π/λ the wave number, $\mathbf{E_{ac}}$ the acoustic energy and **z** the distance from the centre of the channel.

This channel can concentrate cells of sizes between 3 and 40 µm. The maximum reconcentration factor will be x3, and the power needed to attain that factor depends on the size of the cells. The bigger the cell, the lower the needed power.

Cells are injected in the central outlet at a given flow rate, between 5 and 40 ml/min with peristaltic pumps. The channel is 250 µm high and 10 mm wide. The piezo transducer is 50x10 mm. Each outlet has a third of the inlet flow rate. The number of cells in the lateral and central outlets is counted and the yield is determined by the ratio between the number of cells in the central outlet and the sum of all the cells recovered in the 3 outlets.

Cell viability

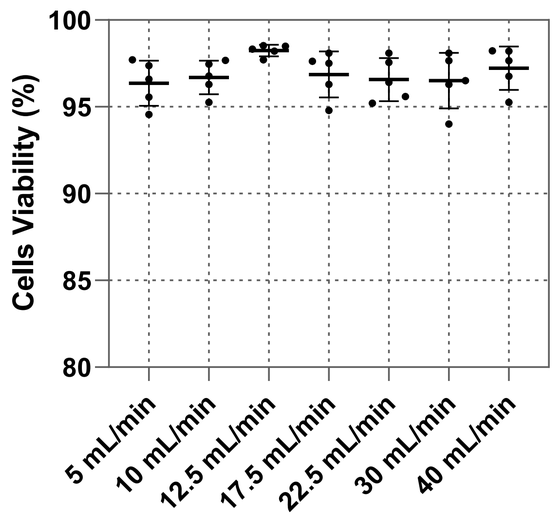


Fig.2: *Jurkat viability after reconcentration.*

No significant viability change is observed on Jurkat cells after exposure to ultrasound, for all flowrates. Power used: 5-30 W (an estimated acoustic energy density in the channel of 30-180 J/m³). Similar viability has been recorded for platelets and red blood cells.

High flow reconcentration of Jurkat and red blood cells

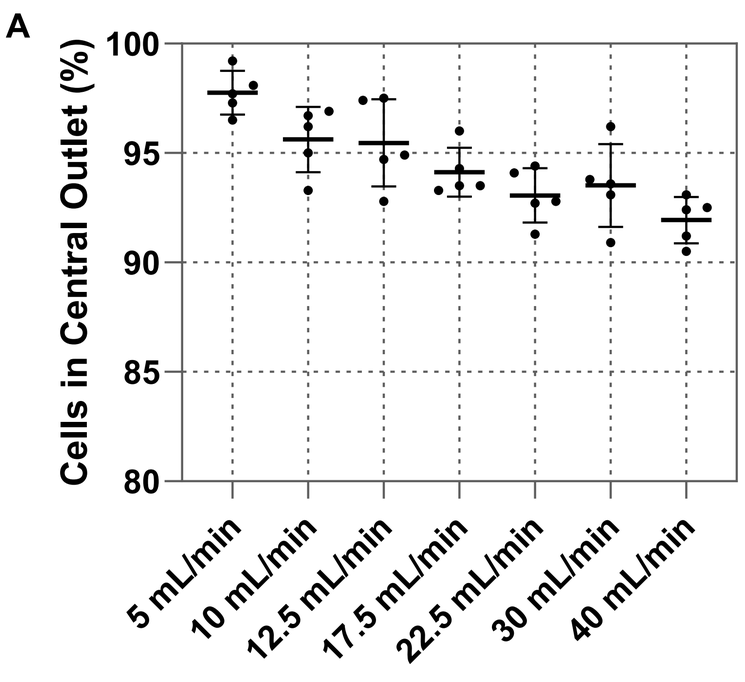
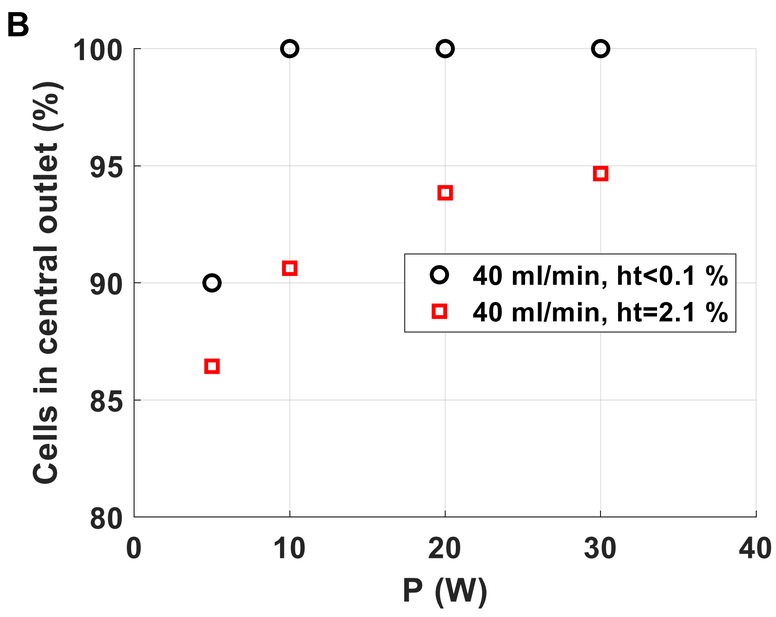


Fig.3: ***Concentration experiments performed on Jurkat and red blood cells.*** **A.** Number of cells in central outlet for 5-40 ml/min, n=5. Initial concentration: 80 000 cells/ml, in 500 ml total volume. **B.** Experiments with a flow rate of 40 ml/min on 2 different hematocrits for diluted red blood cells in Isoton, with powers from 5 to 30W.



Cells passing through the channel are deviated by the acoustic radiation force. At 5 ml/min, 5 W is enough to obtain yields above 95%. The higher the flow rate, the faster the cells move in front of the transducer. This is why a high power (30W) is then needed for 40 ml/min as shown on fig. 3B for red blood cells. Cells that are the closest to the walls of the channels are most likely to get unaffected by acoustophoresis, as the force is 0 on the walls. As a result, higher flow rates give slightly inferior yields to lower flow rates. Yields always stay above 90% nonetheless.

Different applications may require a lower flow rate to keep precious cells, or may require a high flow rate on common cells that need a faster processing time.

Yields above 90% recovery are obtained both for Jurkat and red blood cells at 40 ml/min and a power of 30 W.

Channel

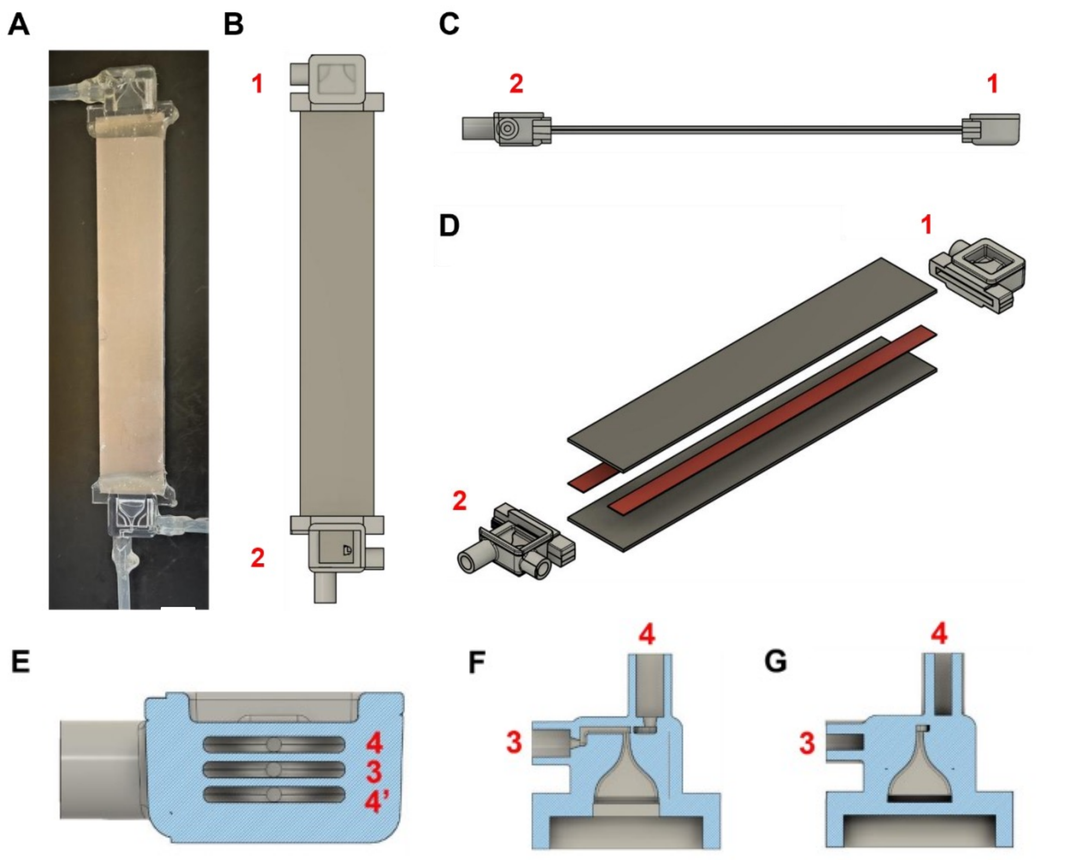


Fig.4: A. Photo of a 1 inlet/3 outlets acoustic channel for cell reconcentration, with 3D printed ends. Scale bar: 10 mm. **B.** CAD drawing of the same channel, front view. **C.** CAD drawing, side view. **D.** CAD drawing, exploded view. Part **1** is the inlet and part **2** is the outlet. Two 250 µm thick PMMA plates (dark red) are sandwiched between two 1 mm thick stainless steel plates (dark grey). **E.** Vertical cut of part **2** showing the flow separation in 3 with 2 lateral outlets (**4** and **4'**) and a central outlet **3** **F.** Horizontal cut of part **2** at the same height level as the central outlet **3**. **G.** Horizontal cut of part **2** at the same height level as the lateral outlet **4**. The lateral outlets **4** and **4'** join as one single channel allowing for the connection to a single tube.

Stainless steel channels allow for high acoustic transmission compared to plastic. The smooth steel finish also prevents cavitation bubbles to appear, leading to a safe handling of cells even at high acoustic power.

Conclusions

No side effects are observed on Jurkat cells after reconcentration at 40 ml/min and 30W. The hemoanalyzer did not detect any apoptosis of red blood cells either, with the same physical parameters.

For flow rates between 5 and 40 ml/min we reach reconcentration yields superior to 92% on average (n=5) with Jurkat cells. This corresponds to a reconcentration factor of x2.8. Experiments with red blood cells showed similar results, with a reconcentration yield above 95% and a factor of x2.85. Channels can then be used in series in order to reconcentrate cells even further, two channels for example having a factor of 2.8x2.8=7.8.

Perspectives

- Different pumps with higher flow rates are being tested, up to 100 ml/min. It is likely that powers up to 50 W will be needed for good yields. A preliminary test with red blood cells (ht=1.0%) at 95 ml/min resulted in a yield of 87% and a reconcentration factor of x2.5.
- This study led to the development of industrialized sterile cassettes consisting of 2 channels that can be used in series to have a concentration factor up to x9, or in parallel, to double the total processing flow rate. The cassettes use the same transducer frequency and materials.

References

1. Bohec P. et al. Acoustophoretic purification of platelets: Feasibility and impact on platelet activation and function. Platelets. 2017 Dec 6:1-7.

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