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Bulk acoustic waves-based system for a rapid and high throughput cell washing

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Introduction

These last decades have seen the emergence and development of cell-based therapies, notably those based on mesenchymal stromal cells (MSCs), human induced pluripotent stem cells (iPSCs) and, CAR-T cells. The advancement of these promising therapies necessitates scaling up the throughput of cell processed for industrialization and lowering the production costs. Among the different cell processes, the cell transfer from one medium to another (e.g., change of cell culture medium, removal of enzymes and viruses, removal of cell debris) is particularly common for the cell manufacturing (1). The classical and most widely used method to remove a medium, and its components, is the conventional centrifugation. However, in addition to inducing high physical stress on the cells, the centrifugation is time consuming and requires operator intervention making this step complicated to integrate into an automated process.

To overcome those drawbacks, the use of acoustic force to manipulate cells (acoustophoresis) has emerged as a promising solution (2). Indeed, acoustophoresis offers the advantages of being a non-contact method, inducing low mechanical stress and, being easily integrated in a controlled and closed fluidic system. All of these qualities allow acoustophoresis to be a good manufacturing practices (GMP) compliant technology and a game-changer in cell manufacturing.

In this work, we developed a system based on bulk acoustic waves allowing the transfer of cells from one medium to another while limiting the transfer of proteins. Moreover, we optimized the flow rate in order to make the throughput of processed cells consistent with the needs of cell manufacturing.

Methods

The experimental device is composed of a piezoelectric ceramic (optimized for a frequency $f = 2.5$ MHz), a liquid-cooling system (regulating the temperature of the system at 20°C) and, a stainless-steel channel (Fig 1). The inner thickness of the channel is 250 μm . The flow rates of the 3 inlets and both side outlets are controlled independently with peristaltic pumps. Red blood cell (RBC) concentrates were obtained by centrifugation of whole blood collected from volunteer informed donors. RBC and albumin were suspended in physiological serum and injected in both side inlets. Physiological serum was injected in the central inlet. The albumin concentration was measured with an albumin assay kit. The RBC recovery was assessed by flow cytometry.

Results

The experimental process is shown in Fig 1. We investigated different flow rate configurations in order to maximize the efficiency of the separation: side flow rate $Q_S >$ central flow rate Q_C , $Q_S = Q_C$ and, $Q_S <$ Q_C . The separation efficiency of albumin and RBC was higher for $Q_S <$ Q_C and lower for $Q_S >$ Q_C (mean = 1.410 ± 0.092 vs mean = 1.114 ± 0.063 respectively, $p=0.007$, $n=4$). Then, we applied a flow rate configuration $Q_S = 0.75$ mL/mn and increased Q_C up to 12 mL/mn. The separation efficiency was positively correlated to Q_C (Fig 2A). The mean of albumin removal increased with Q_C to reach a mean = 89.1 % (± 0.6 , $n=4$) with $Q_C = 12$ mL/mn. Despite the lack of significant difference, the RBC recovery tends to be more efficient at $Q_C = 6$ mL/mn with a mean of RBC = 87.7% ± 2.9 . Finally, we applied a central flow rate $Q_C = 9$ mL/mn and increased the throughput with Q_S up to 6 mL/mn. The separation efficiency was negatively correlated to Q_S and was maximal for $Q_S = 0.75$ mL/mn.

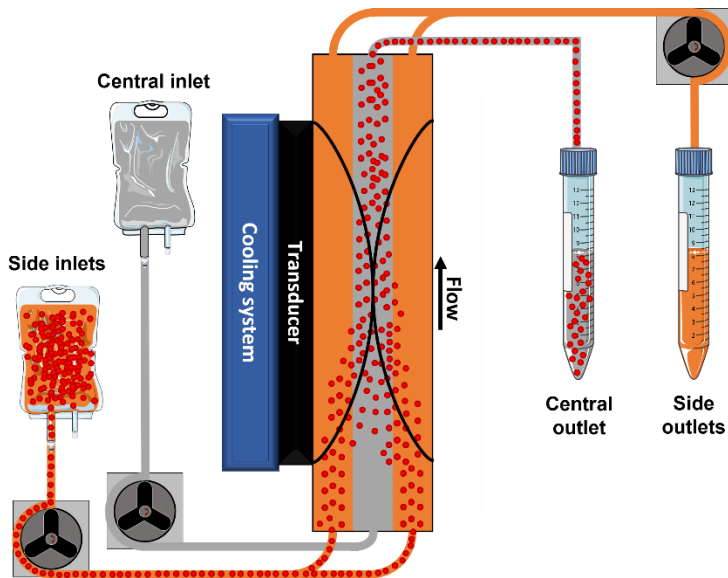


Figure 1: Schematic representation of the bulk acoustic waves-based cell washing setup. RBC and albumin solution are injected in the side inlets. While acoustic is turned on, most of the cells are recovered in the central outlet while the majority of the albumin remains in the side outlets.

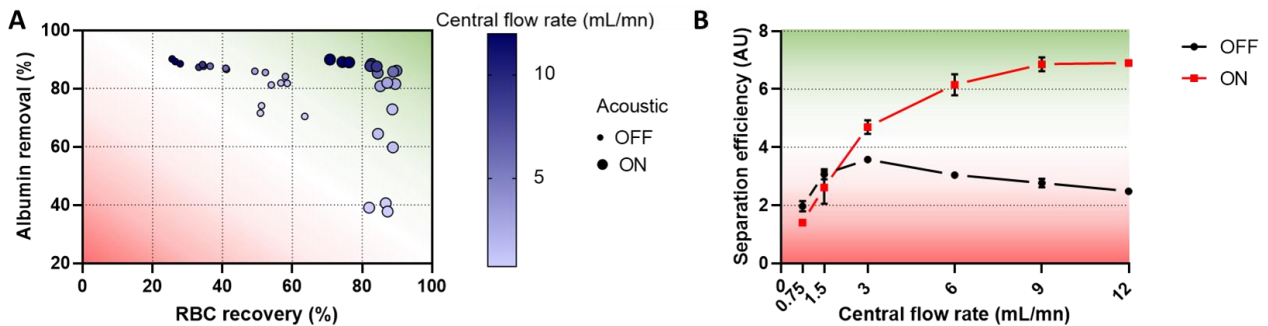


Figure 2: Cell washing efficiency depending on the central flow rate. **A** RBC recovery and albumin removal depending on the central flow rate. Side flow rate was 0.75 mL/mn, central flow rate was 0.75 to 12 mL/mn, input power was 8 W, n=3. **B** Separation efficiency of RBC and albumin depending on the central flow rate. The separation efficiency (Arbitrary Unit) was calculated as a ratio of RBC recovery and albumin concentration in the central outlet. The black and red lines represent the separation while the acoustic is turned off and on, respectively.

Perspectives

Next steps will be to investigate the impact of higher cell and protein concentrations on the separation's efficiency. We plan to use a loop system to expose cells to acoustics twice in order to increase our separation efficiency and to reiterate those results with other cell types. Finally, we will study the impact of the process on both the cells' morphology and viability.

Conclusion

In this study, we developed an acoustic device for cell washing allowing the transfer of cells from one medium to another and the removal of proteins.

We optimized the flow rates in order to remove nearly 90% of the proteins and recover 83% of the cells in a single passage through our system with an input flow rate of 0.75 mL/mn. These results highly suggest that cell washing based on acoustophoresis is a relevant and promising solution for numerous applications in cell manufacturing.

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