Acoustic Beads: A novel method for cell sorting using acoustophoresis

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Background and Aim

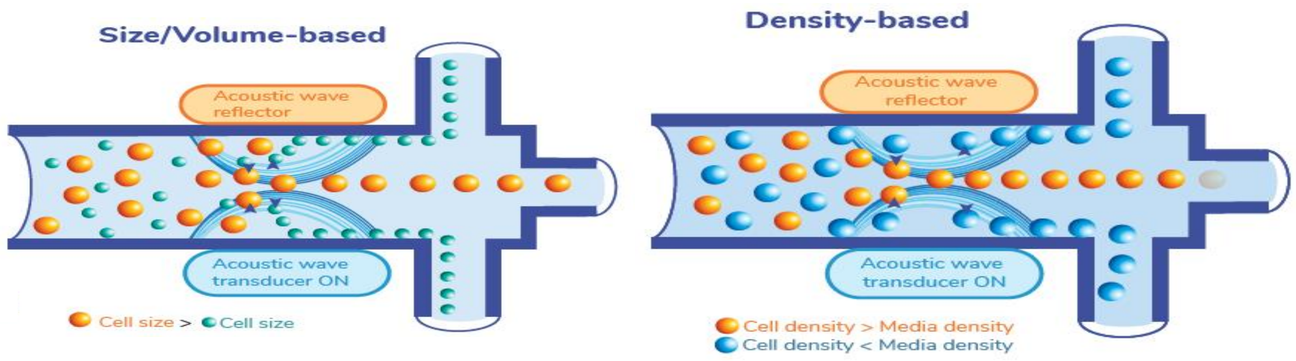
Rare cell sorting is a critical step in bioprocessing as cells are often exposed to various environmental factors that can affect their growth, differentiation, and overall behaviour. Acoustophoresis, a non-invasive technique using acoustic radiation forces, offers a gentler and more precise alternative to conventional cell manipulation methods. In a millifluidic channel, acoustic radiation forces act on cells within a continuous flow, guiding them toward the centerline of the channel, where they can be efficiently collected. Notably, this directional cell "migration" can be reversed, redirecting cells to lateral outlets, if their density is lower than the surrounding medium, enabling density-based sorting. This density inversion can be achieved by attaching tailor-made low-density microbubbles (MB) or acoustic beads—to the cells. Of notice, non-functionalized lipid-shelled gas-encapsulated microbubbles which are clinically approved and commercially available as contrast agents in ultrasound medical imaging, making these acoustic beads a practical and scalable tool for advanced cell sorting applications.

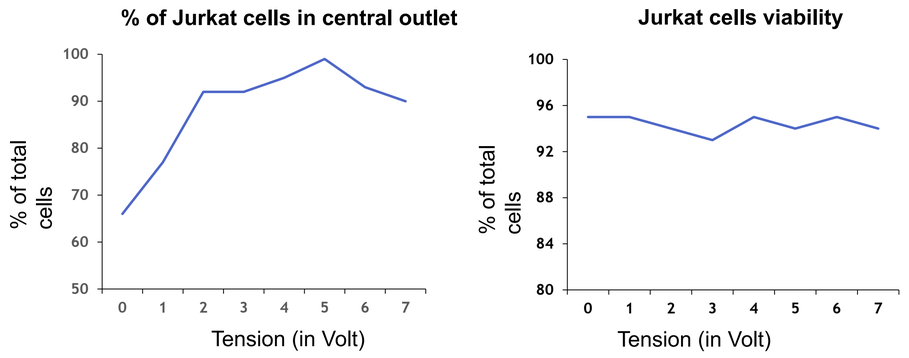
Methods

Acoustophoresis System

How it works

When acoustic waves interact with an object in suspension, they exert a force known as the acoustic radiation force. The direction and magnitude of this force are influenced by factors such as the acoustic wave properties, the physical features of the object (e.g., size, density, shape) and the medium characteristics. This force levitates cells and particles, causing them to move at varying speeds or along different trajectories.



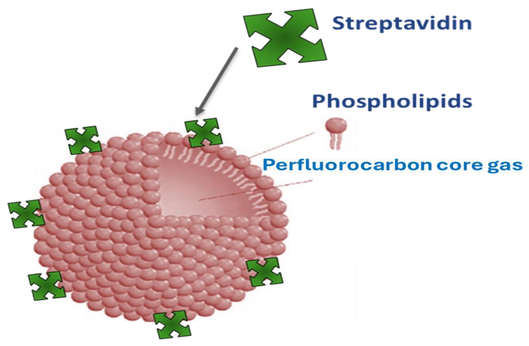


Low pressure < 1-1,5 MPa

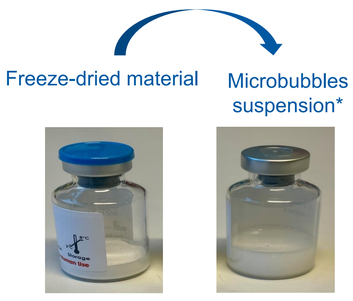
In continuous flow → 1-40 mL.min^{-1}

Contactless

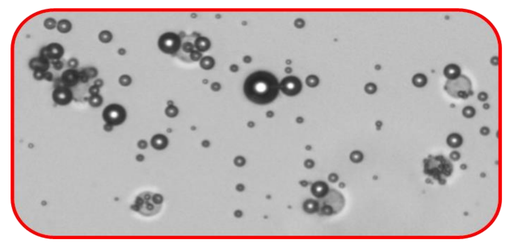
Microbubbles Technology



Schematic representation of microbubbles



***Already used in diagnostics**

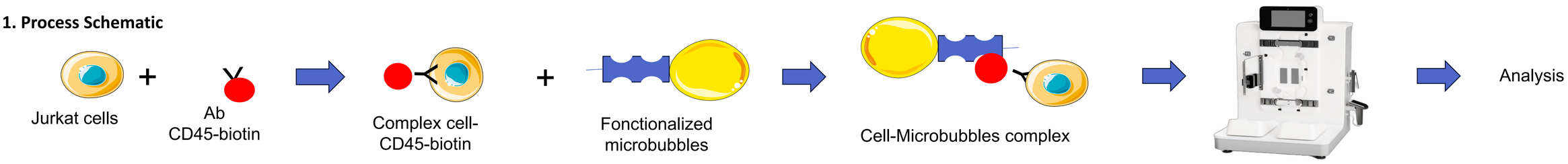


Bright field image of microbubbles

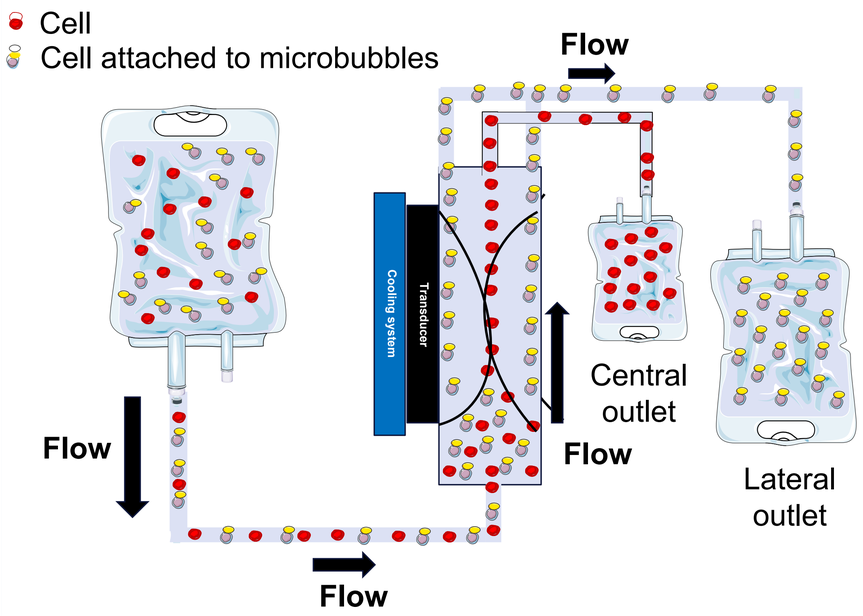
- Streptavidin conjugated microbubbles, consisting **of lipid-shelled, gas-encapsulated microbubbles** specifically designed and optimized for cell sorting applications
- **Long-term stability** of the freeze-dried product is demonstrated, maintaining effectiveness for up to 3 years at 5°C
- Upon reconstitution with a saline solution, microbubbles are generated, and **the suspension remains stable when stored at 5°C**
- **Product** is ready for **production for clinical use**

Results

1. Process Schematic

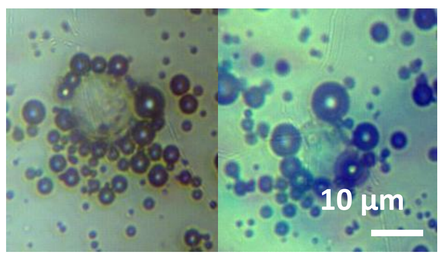


2. A combination of both technologies enhances the precision and efficiency of the cell sorting process

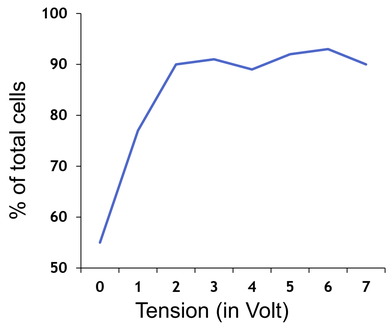


3. Evaluation of stability of cell-microbubbles complexes under acoustic

Microbubbles are Stable under Acoustic Stimulation



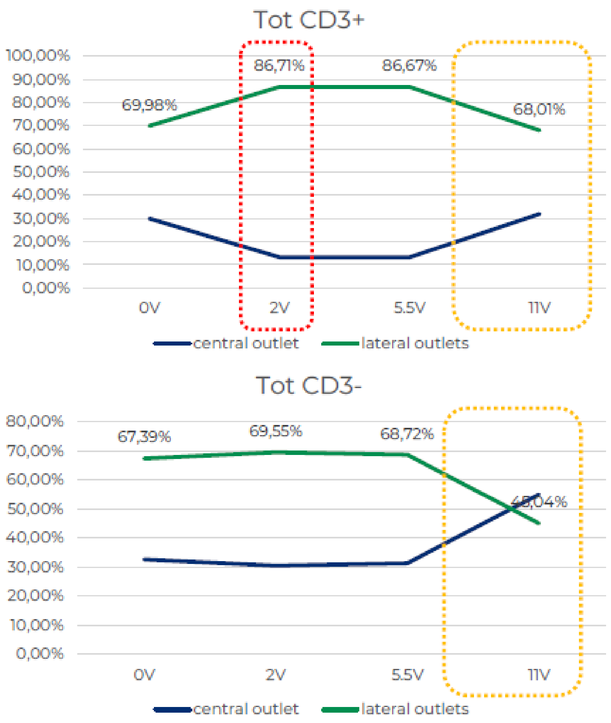
Bright field image of Jurkat cells after incubation with MB



Cells Attached to Microbubbles Remain Stable under Acoustic Stimulation, with no Impact on Cell Viability

Up to 92% of Jurkat Cells are Successfully Sorted to the Lateral Outlet

4. CD3 cell sorting from PBMC



86% of CD3+ cells were collected in the lateral outlet

Conclusion

Combining proprietary **acoustophoresis** technology with low-density **microbubbles** represents a novel**, gentle, and effective cell-sorting** solution. This approach improves cell viability in **continuous flow, operates contact-free**, and is compatible with **GMP** standards. Implemented within a **closed and automated system**, it also **enables cost-effective and scalable processing**, making the **large-scale sorting of rare and complex cells** faster, gentler, and broadly accessible.

Future Directions

Parameters Optimization :

- Purity
- Repeatability
- Number and Size of microbubbles per cell
- Processing time (1-40 mL/min)
- Microbubble removal

Future Applications :

- Leukopak
- Functionality