CUTTING-EDGE LIPID-BASED MICROBUBBLE PLATFORM: ENHANCING CELL SORTING

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LIFE FROM INSIDE

BACKGROUND & AIM

Innovative cell therapies face practical barriers for proper clinical implementation, in part due to labor-intensive cell manufacturing processes that lack flexibility, consistency and scalability. **Separation/isolation of target cells** from other contaminants is **essential to streamline** the cell therapy workflow.

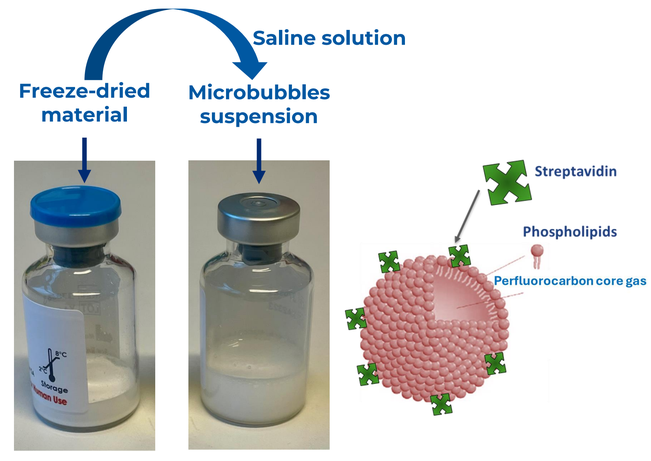
Magnetic-Activated Cell Sorting (MACS) is currently the industry standard for cell isolation but suffers from some limitations, such as scalability and cost-effectiveness.

Herein, we propose a **versatile and optimized** streptavidin (STV) conjugated **lipid-shelled microbubbles** (MB) to be combined to any biotinylated ligand as an alternative cell sorting technology. Using the natural **MB floatability/buoyancy**, targeted cells can be **gently and efficiently sorted** from complex biological matrices.

METHODOLOGY

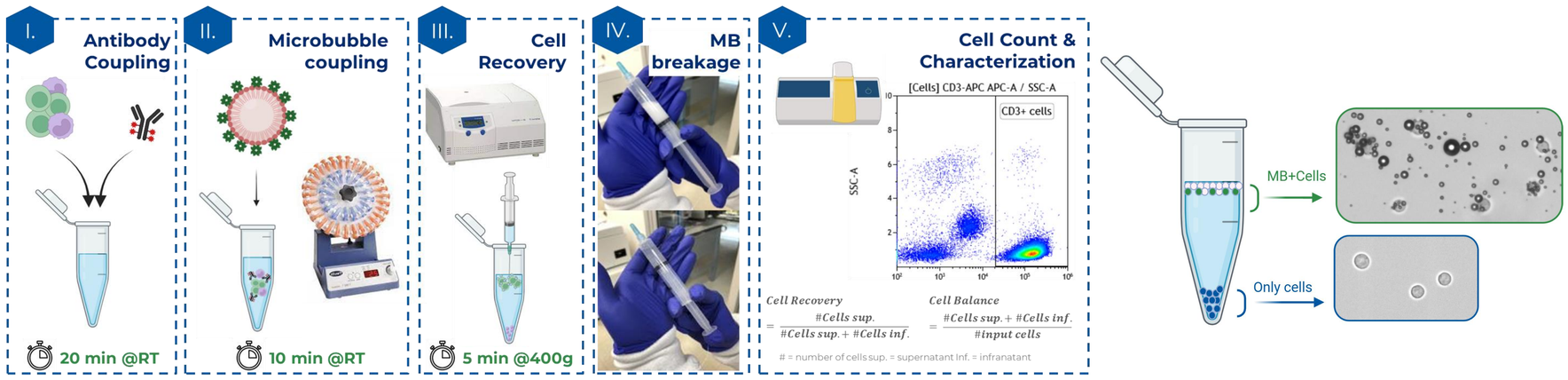
1) MICROBUBBLE PREPARATION

- Streptavidin conjugated microbubbles, consisting of a gas encapsulated in a lipid shell, were designed and optimized for cell sorting purposes
- A robust and scalable manufacturing process was established allowing 1500 vials / lot
- Long-term stability of the freeze-dried product is demonstrated (3-y @ 5°C)
- Upon reconstitution with a saline solution, microbubbles are generated and the suspension is stable upon storage at 5°C
- Ready for GMP production for clinical use



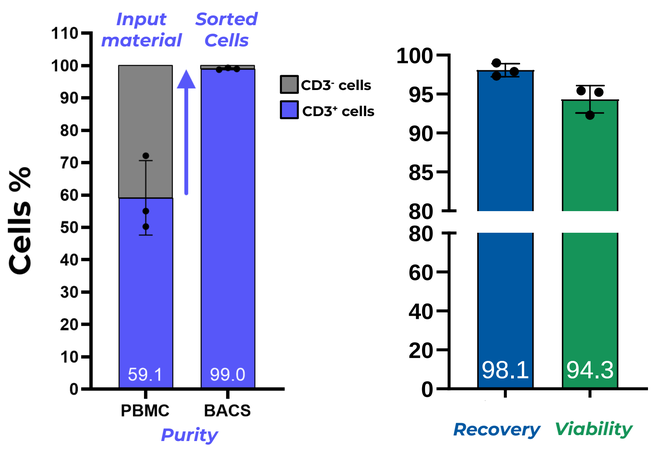
2) CELL SORTING

Proof-of-principle was performed by pre-incubating 5x10^6 Peripheral Blood Mononuclear Cells (PBMC) with biotinylated anti-human CD3, CD4 or CD8 IgG before incubation with STV-conjugated MB. **Gentle isolation of target cells** was achieved after centrifugation and isolated cells and PBMC were characterized by flow cytometry.



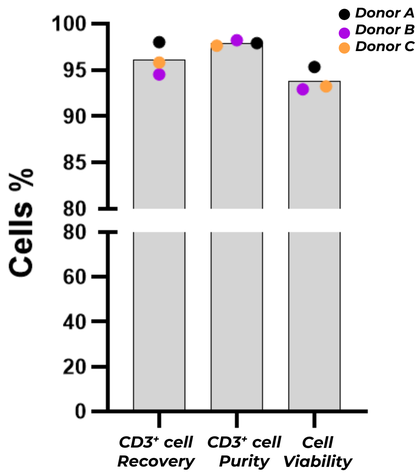
RESULTS

T-cell isolation using STV-conjugated Microbubbles, anti-human CD3 Antibody and Peripheral Blood Mononuclear Cells (PBMC)



- CD3+ cells capture was performed by pre-incubating 5x10^6 PBMC with a specific biotinylated anti-human CD3 IgG before incubation with STV-conjugated MB.
- **Gentle isolation** of target cells was achieved after centrifugation leading to highly purified cells (>98%) with **high recovery efficiency and cell viability**

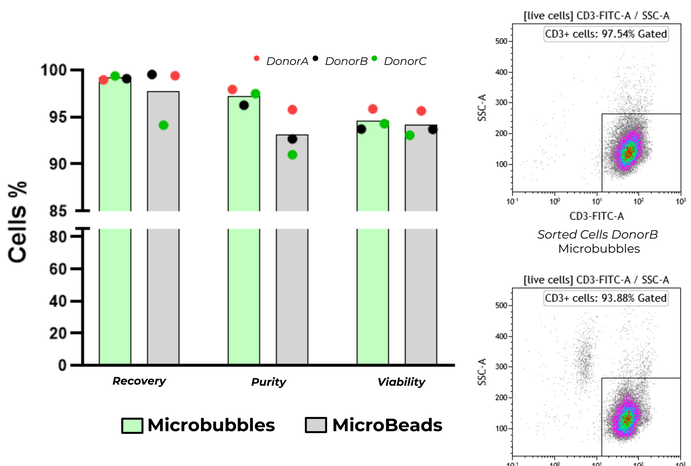
T-cell isolation using STV-conjugated Microbubbles, anti-human CD3 Antibody and 10th size Frozen Leukopak



Proof-of-concept: STV conjugated MBs ability to efficiently capture CD3+ cells was further demonstrated using clinically relevant starting material, namely **frozen leukopak**.

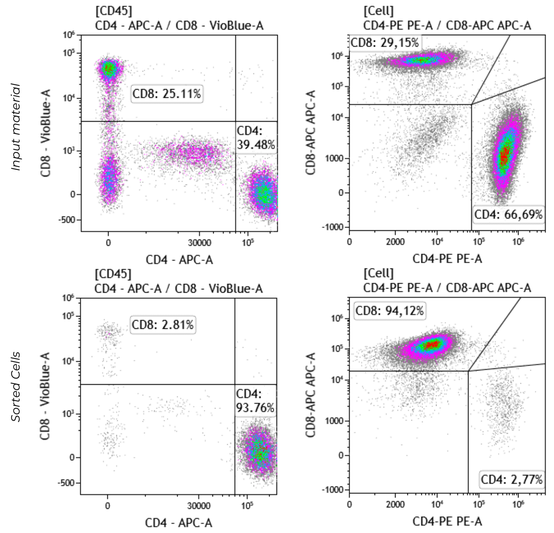
- 9x108 WBC (3 different donors) were pre-incubating with biotinylated anti-human CD3 IgG before incubation with STV-MB followed by a gentle centrifugation.
- Here again, excellent performances were obtained in terms of **CD3+ cell recovery, cell purity and cell viability.**

T-cell isolation using STV-Microbubbles or STV-Beads, anti-human CD3 Antibody and Peripheral Blood Mononuclear Cells (PBMC)



- **Comparison to Benchmark** was performed by pre-incubating 1x10^7 PBMC with biotinylated anti-hu CD3 IgG before incubation with either STV-MB or STV-beads (#130-048-102 Miltenyi).
- MACS was performed manually with MS column (#130-042-201 Miltenyi) following manufacturer's instructions.
- **Similar or superior performances** to current market standards were achieved in terms of cell recovery, viability and purity.

CD8+/CD4+ cell isolation using STV-conjugated Microbubbles, anti-human CD8 and/or CD4 Antibodies



- **CD3+ subpopulations** such as CD8+ (lower right panel) or CD4+ (lower left panel) can be **efficiently isolated** from either PBMC or T cells with biotinylated anti-hu CD8 or CD4 IgG before incubation with STV-MB.
- Thanks to the high versatility of STV-MB, subpopulations can be **isolated separately or conjointly**.
- By applying over-pressure on sorted cells, Microbubbles can be **easily eliminated**, allowing downstream applications such as **cell activation or multiple rounds of cell selection**.

CONCLUSIONS

- Our innovative and cost-effective **lipid-based MB** platform presents **competitive benefits** over existing cell sorting systems (cell **purity, recovery** and **viability)**
- Our platform is **highly versatile**, can be used for **sequential rounds of positive and negative** cell selection for a **wide variety** of cell types, including NK and stem cells
- The process is **scale-independent**, **bead-free**, space efficient and easily amenable to **automated** cell therapy manufacturing purposes
- Thanks to the **gentle manipulation**, our **traceless reagent** will improve the quality/fitness of the cellular material and ultimately define the **therapeutic outcome**