TRANSFORMING CELL SORTING WITH LIPID-BASED MICROBUBBLE INNOVATION

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BACKGROUND & AIM

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

Magnetic-Activated Cell Sorting (MACS) is the current industry standard for cell isolation but suffers from some limitations, such as scalability, cost, cell recovery/viability and difficulty to remove solid beads. Lipid-stabilized microbubbles (MB) are widely used in the clinics for decades as contrast agents for numerous indications and can be functionalized to address cell sorting challenges.

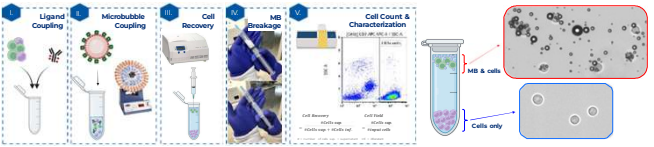
Herein, we introduce a modular and optimized streptavidin (STV) conjugated microbubble reagent that can be combined with any biotinylated specific ligand as an alternative cell sorting technology. By leveraging the natural buoyancy of MB, targeted cells can be gently and efficiently separated from complex biological matrices in a cost-effective and simple way.

METHODOLOGY

1) MICROBUBBLE PREPARATION

- Streptavidin (STV) 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 (4-y @ 5°C).
- Upon reconstitution with a saline solution, microbubbles are generated, and the suspension is stable upon storage at 5°C.
- The BACS MB reagent is under development for clinical use.

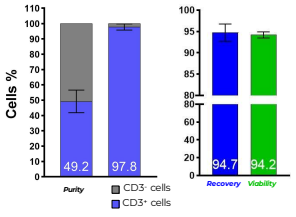
2) BUOYANCY-ACTIVATED CELL SORTING (BACS)



Standard workflow: cells (e.g. PBMC) are mixed with the desired biotinylated ligand (e.g. anti-human CD3, CD4 or CD8 IgG) before incubation with STV-conjugated MB. Gentle isolation of target cells is achieved after centrifugation and MB can be deflated by applying overpressure. Isolated cells and the starting material are characterized by flow cytometry.

RESULTS

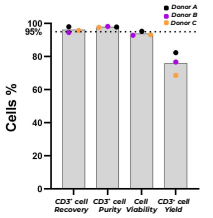
T-cell isolation using STV-conjugated Microbubbles, anti-human CD3 Antibody and Peripheral Blood Mononuclear Cells (PBMC).



Proof-of-principle

- $CD3^+$ 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 (7-AAD-).

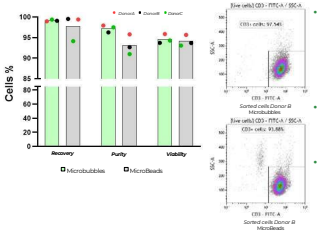
T-cell isolation using STV-conjugated Microbubbles, anti-human CD3 Antibody and 10th size Frozen Leukopak.



Proof-of-concept

- STV-conjugated MBs ability to efficiently capture $CD3^+$ cells was further demonstrated using clinically relevant starting material, namely frozen leukopak.
- $9x10^8$ WBC (3 different donors) were pre-incubated 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.

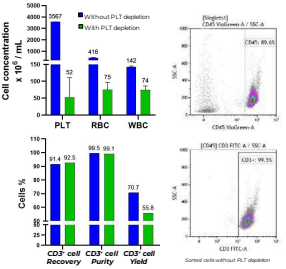
T-cell isolation using STV-conjugated Microbubbles or STV-Beads, anti-human CD3 Antibody and Peripheral Blood Mononuclear Cells (PBMC).



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 standard (MACS) were achieved in terms of cell recovery, viability and purity.

T-cell isolation using STV-conjugated Microbubbles, anti-human CD3 Antibody and unprocessed fresh Leukopak.



Straightforward & column-free

- Low-speed centrifugation can remove platelets (PLT) but increases processing time and causes significant cell loss.
- Starting from fresh, unprocessed leukopaks (~$9x10^8$ WBC), excellent $CD3^+$ cell isolation performances were obtained with similar purities and recoveries compared to platelet-depleted samples.
- This time-saving procedure led to a higher yield, without any risk of column cloggings.

CONCLUSIONS

- Our innovative, **cost-effective**, space efficient and versatile **lipid-based MB** platform presents **competitive benefits** over existing cell sorting technologies.
- The target cells can be efficiently collected with a **high purity** along with a very **good recovery** and with preserved **cell viability**.
- The process is **scale-independent**, **column-** & **bead-free** and easily amenable to **automation** to fulfill cell therapy manufacturing requirements.
- Thanks to the **gentle manipulation**, our **innovative reagent** will improve the quality/fitness of the cellular material and ultimately define patient **therapeutic outcomes.**

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