ONE PLATFORM, MANY TARGETS: TRANSFORMING CELL SELECTION AND ACTIVATION USING LIPID-BASED MICROBUBBLE

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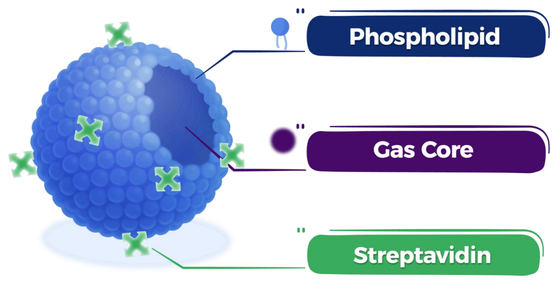
BACKGROUND & AIM

Over the past decade, **cell therapies** have dramatically transformed the immune-oncology landscape, expanding far beyond hematologic cancers to tackle more complex indications such as solid tumors, autoimmune and neurodegenerative diseases, as well as age-related conditions. Despite these advancements, improving accessibility and affordability continues to be one of the industry's most pressing challenges. Robust, scalable, and large-scale manufacturing capabilities have become essential, alongside extremely precise and efficient **cell isolation technologies**.

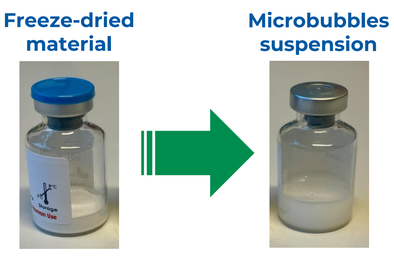
To this aim, we have developed **BACSA (Buoyancy-Assisted Cell Sorting and Activation),** a cutting-edge technology designed to enable highly efficient cell selection and activation. BACSA stands out by streamlining workflows, significantly reducing manufacturing costs, and opening the door to large-scale patient access. This innovative approach not only surpasses traditional methods but also paves the way for broader implementation and greater impact within the field.

Herein, we propose our versatile cell selection and activation **BubbleGen™** platform based on Bracco's long-standing use of microbubble technology in the clinical diagnostic imaging space. Using the natural **buoyancy**, of streptavidin conjugated microbubbles, targeted cells can be **gently and efficiently sorted** from complex biological matrices.



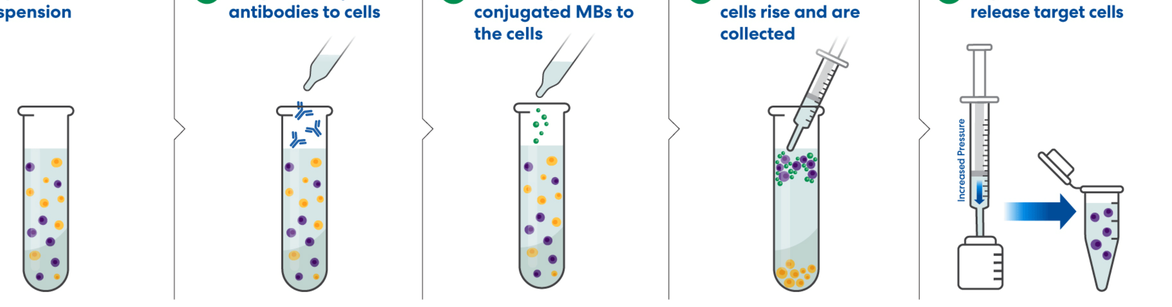
METHODOLOGY

1) MICROBUBBLE PREPARATION



- **BubbleGen™ Streptavidin** conjugated reagent, consisting of gas filled lipid-shelled microbubbles was designed and optimized for cell sorting purposes
- **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-compliant production** for clinical use

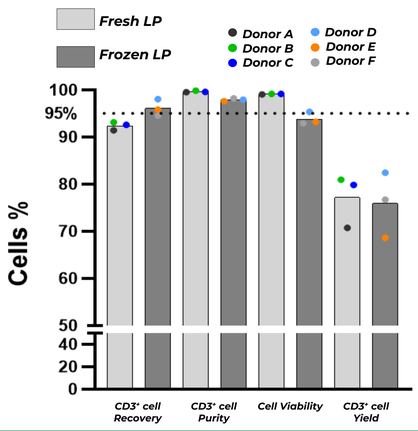
2) CELL SORTING



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 streptavidin conjugated microbubble. **Gentle isolation of target cells** was achieved after centrifugation and isolated cells and PBMC were characterized by flow cytometry.

SELECTED APPLICATIONS

Positive T-cell isolation using anti-human CD3 Antibody and 10th size fresh and frozen Leukopaks (9x10^8 WBC)



- **Significant enrichment** of CD3+ cells after anti-CD3 BACS

→ High purity of CD3+ T-cell (≥98%)

→ High efficiency of human CD3+ cell recovery (>90%)

→ Excellent viability of isolated cells starting from fresh leukopak (~99%)

Positive isolation: *CD3+ cells*

Rare cell enrichment: *Stem cells*

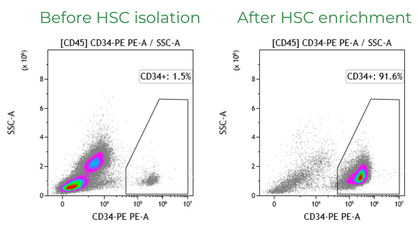
Negative isolation: *CD3+ cells*

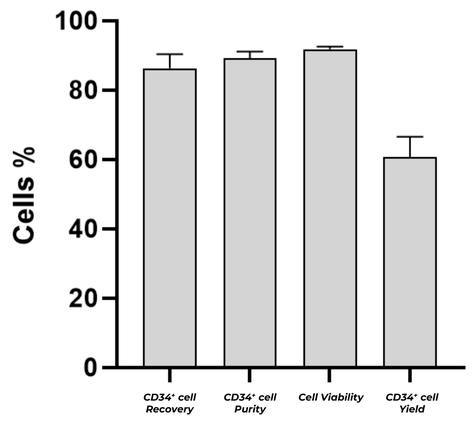
Sequential separation: *CD4/CD184*

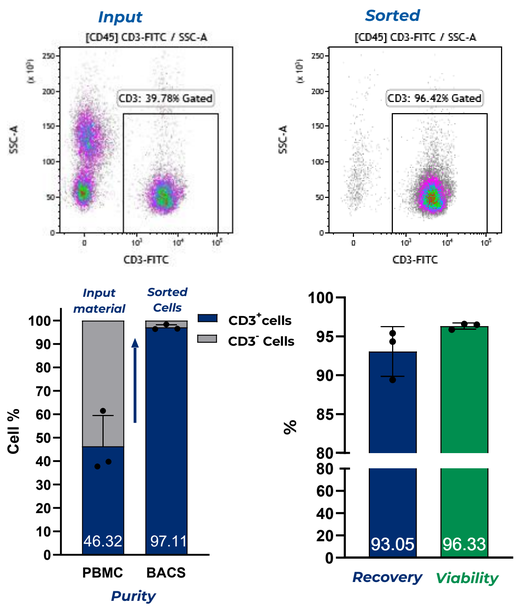
Stem cell isolation using anti-human CD34 Antibody and frozen mobilized apheresis (9x10^8 WBC)

- **Significant enrichment** of Hematopoietic Stem Cells after anti-CD34 BACS

→ Purity ~90%
→ Recovery >85%



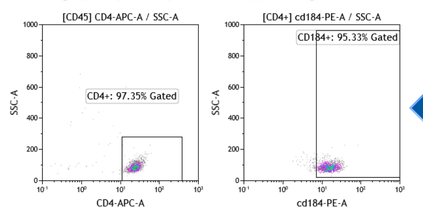




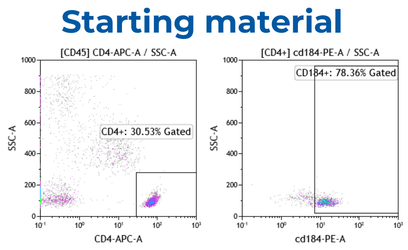
- **CD3+ cells capture** was performed by pre-incubating 5x10^6 PBMC with a specific biotinylated anti-human antibodies cocktail before incubation with Streptavidin microbubbles.
- **Gentle and Consistent isolation** of target cells was achieved after centrifugation leading to highly purified cells (>97%) with **high recovery efficiency and cell viability**

Negative T-cell isolation from PBMC using an optimized anti-human antibodies cocktail

Sort from PBMC

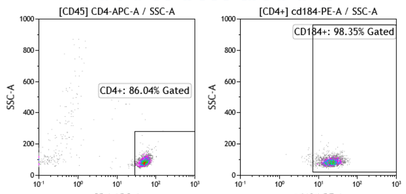


Starting material



- **Starting material: Patient whole blood**
- **2-step cell sorting**
  - Positive selection of CD4+ cells using biot anti-CD4
  - Microbubble deflation and positive selection of CD184+ cells using biot anti-CD184
- **Results**
  - Straightforward approach without Ficoll step
  - Target cells efficiently collected from whole blood
  - Higher purity (95%) with BACS from PBMC.

Sort from blood



CD4/CD184 sequential isolation: Microbubbles enable multiple subsequent positive isolations using different markers

CONCLUSIONS

- Our **BubbleGen™** platform based on gas filled lipid-shelled microbubbles does not require magnetic beads, columns, or hollow glass microspheres; the microbubbles use **biocompatible materials and can be deflated**.
- Our technology enables efficient and flexible selection strategies including **positive, negative and sequential approaches** to address sensitive or difficult-to-isolate cell populations
- Performances are demonstrated across a large variety of starting materials and cell types including **T cells, NK cells and stem cells** for different therapeutic applications.
- Our platform is **fully compatible** with automated and scalable manufacturing systems, with the potential to **unlock new possibilities**—from research and development all the way through to clinical and commercial applications.