MODULAR MICROBUBBLE REAGENT: EFFICIENT AND SIMULTANEOUS CELL SELECTION AND ACTIVATION

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LIFE FROM INSIDE

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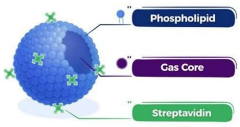
BACKGROUND & AIM

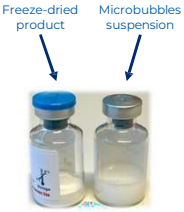
Innovative cell therapies face significant hurdles in clinical implementation, primarily due to labor-intensive cell manufacturing processes that lack flexibility, consistency and scalability. Efficient isolation of target cells from contaminants is critical to streamline workflows. Besides selection, current T cell activation methods rely on magnetic beads or polymer-based reagents. However, these approaches suffer from limitations in scalability, cell recovery/viability, cost and complexity of beads removal. Lipid-shelled microbubbles (MB), widely used as ultrasound contrast agents in clinical practice, can now be functionalized to overcome these challenges in T cell selection and activation.

We present a novel approach for simultaneous selection and activation of target cells using the BubbleGen™ Streptavidin-conjugated MB, designed to pair with any biotinylated ligand, so-called Modular MB Reagent. By harnessing the natural buoyancy of MB, targeted cells can be gently and efficiently separated while undergoing activation. This versatile Modular MB Reagent is GMP-ready to facilitate a smooth transition to clinical use.

METHODOLOGY

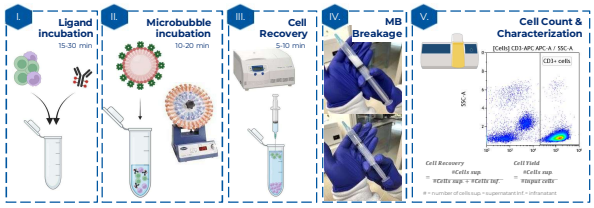
1) BubbleGen™ Streptavidin Microbubble Reagent Preparation





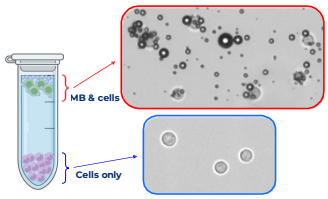
- BubbleGen™ reagent consisting of streptavidin-conjugated, lipid-shelled Microbubbles was designed and optimized for cell sorting purposes.
- Long-term stability of the freeze-dried product is demonstrated (3-y @ 5°C).
- Upon reconstitution with a saline solution, microbubbles are generated, and the suspension is stable up to 3 months upon storage at 5°C.
- BubbleGen™ MB reagent is under development for clinical use.

2) Simultaneous T Cell Selection and Activation

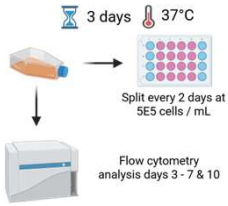


Standard workflow:

- (I.) cells (e.g. PBMC) are mixed with specific biotinylated anti-human CD3 and CD28 antibodies
- (II.) modular MB reagent is added to the prelabeled cells and incubated under agitation.
- (III.) gentle isolation of target cells is achieved after centrifugation and (IV.) MB can be deflated by applying overpressure.
- (V.) cells are counted and characterized



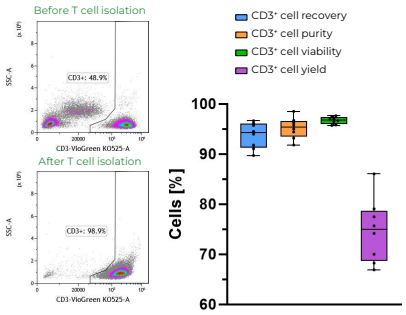
3) T Cell Expansion and Characterization



- Activated Human T cells are seeded at 5 x 10^5 in RPMI + 10% FBS + IL-2 (30IU/mL) and cultured for 3 days at 37°C, 5% CO_2.
- Cells are subsequently subcultured every 2 days.
- Cell viability along with activation/exhaustion markers are measured 3, 7 and 10 days post-activation.

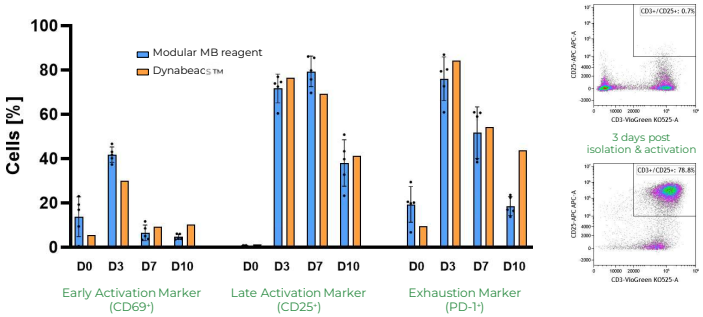
RESULTS

T cell isolation using BubbleGen™ MB reagent and anti-human CD3&CD28 biotinylated antibodies.



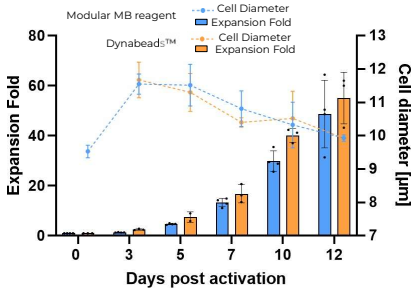
- **T cell isolation** was performed by pre-incubating 1 x 10^7 PBMC with biotinylated anti-hu CD3 IgG and anti-hu CD28 IgG before incubation with Modular MB reagent.
- Significant **enrichment of target cells** was obtained with **~95% purity** after isolation and with **high viability (>95%)**.
- **High cell recovery** (>90%) along with **good yield** (>70%) was achieved using the Modular MB reagent and starting from frozen material.

T Cell Activation and Exhaustion



- Percentages of CD69, CD25 or PD-1 positive T cells were extracted from flow cytometry data.
- **The proportions of activated** (CD25+) **T cells** were similar 3-, 7- and 10-days post-activation using Modular MB reagent or Dynabeads ™ Human T activator CD3/CD28 (Gibco ™, #11131D).
- Transient expression of the PD-1 (aka CD279) **exhaustion marker** was observed with a peak of positive cells at D3 and a percentage back to basal level at D10.

T Cell Expansion



- 3 days after isolation and activation using Modular MB reagent, mean **cell diameter** increased (9.5 to 11.5 µm) confirming **blastogenesis**, which supports rapid proliferation.
- T cell expansion was **donor-dependent**.
- **Further optimization** of the process is currently ongoing to accelerate *in vitro* proliferation.

CONCLUSIONS

- Our proprietary **BubbleGen™** microbubble platform, **cost-effective lipid-based MB Reagent** offers distinctive advantages over conventional cell selection and activation systems.
- The target cells can be **efficiently** and **simultaneously collected and activated** with similar performances than benchmarks.
- The process is **scalable**, **bead- and column-free** and easily adaptable to automated cell therapy manufacturing purposes.

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Learn more at www.Bracco.com/CellTherapy