Manufacturing of "MSC 2.0" Using Functionally Closed Systems: Corning® CellSTACK® and HYPERStack® Vessels

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Introduction

Despite significant advancement in modern medicine, the global prevalence rate of cancer continues to rise. The emergence of chimeric antigen receptor T cell (CAR-T) therapy has positioned cell and gene therapies as promising treatment modalities for various cancers. Aside from T cells and NK cells, other cell types such as mesenchymal stem/stromal cells (MSCs) are actively investigated as potential treatments.

Over the years, it has become increasingly evident that deploying naïve MSCs as anti-cancer therapies has limited efficacy and potentially pose safety concerns, particularly due to their potential pro-tumorigenic effects in certain contexts. Due to their natural tumor-tropism, MSCs are well-suited as targeted delivery vehicles for cancer therapy. A promising strategy to leverage this trait involves genetically engineering MSCs to transport and release anti-cancer agents directly within the tumor microenvironment, thereby reducing systemic toxicity. A major hurdle in advancing engineered allogeneic MSC therapies to clinical use is the challenge of producing cells at a scale that meets clinical demand.

While sufficient for early-stage clinical trials, typical open-system setups present higher contamination risk and labor demand than closed-system setups do. These limitations underscore the urgent need for more integrated, closed, and automated solutions to ensure the clinical and commercial scalability of engineered MSC therapies.

To overcome these limitations and move toward scalable clinical production, AGeM Bio, in collaboration with Corning, has successfully demonstrated the feasibility of scale-out manufacturing of non-virally engineered MSCs (MSC 2.0) using a functionally closed system, Corning HYPERStack vessel. The Corning HYPERStack vessel also presents a small footprint, which is efficient and practical for scalable manufacturing at later phases of clinical trials.

Methods and Materials

Human AD-MSCs, obtained from a healthy donor, at passage 3, were thawed from cryopreserved bags and seeded at a density of 2000 cells/cm^2 into 2-layer Corning CellBIND® surface treated CellSTACK vessel (Corning 3310) containing 250 mL of proprietary media. After 4 days of incubation in a humidified, 5% CO_2 incubator at 37°C, cells were harvested with TrypLE™ Express Enzyme (Thermo Fisher 12604021) and neutralized with an equal volume of proprietary media. Cells were then counted and a volume equivalent to 15 million cells was obtained and topped up to 1300 mL with media. The mixture was transferred into a CellBIND HYPERStack 12-layer vessel and allowed to expand for an additional 3 days. Spent media was then drained and replaced with fresh media supplemented with transfection reagents (including proprietary mix of transfection enhancers together with the gene of interest CD::UPRT::GFP). Cells were incubated for an additional day before they were harvested and assessed for yield, viability, and expression of gene of interest. Cytotoxicity was measured by MTS to assess the anti-tumor effects.

Results and Discussion

After the transfection process, 2.7 x 10^8 modified MSCs (4.5 x 10^5 cells/cm^2) were harvested at a viability and transfection efficiency of 92.9% (measured by dye exclusion) and 47.7% (GFP+ cells assessed by flow cytometry), respectively. Based on previous experience with this transfection model, the expression and accumulation of the reporter will increase over several days of culture, making it more detectable. Hence, cells harvested from the HYPERStack vessel were replated onto a 6-well plate to monitor changes in GFP expression over time.

Our results show that transfected MSCs reached peak transgene expression on Day 2 post-replate, at 79.7%. Notably, transfection efficiency stays relatively stable between 75% and 80% throughout the replate duration (Figure 1).

To evaluate the anti-cancer efficacy of engineered MSCs harvested from the HYPERStack vessel, we conducted a cytotoxicity assay against the drug-resistant glioblastoma cell line U-87MG. This cell line has been adapted for resistance against temozolomide at 40 µM, hence named U-87MGTMZR40. Tumor cells (2500) were seeded per well in a 96-well microplate and allowed to adhere for 5 hours. Engineered MSCs were then added to the cancer cell culture in ratios of 1 MSC to 5, 10, 50 and 100 cancer cells. 7 days later, cancer cell survival was measured by MTS assay. Relative to the untreated control, a ratio of 1 MSC to 100 cancer cells resulted in over 30% tumor cell destruction, while increasing the ratio to 1 MSC per 10 cancer cells led to the elimination of more than half of the tumor population (Figure 2). These findings confirm that the engineered MSCs retain anti-cancer properties and demonstrate that the HYPERStack vessel is a viable platform capable of producing hundreds of millions of cells – sufficient to support clinically meaningful dosing.

Further refinement of the reagent composition, and culture condition could potentially enhance the yield of these engineered MSCs further.

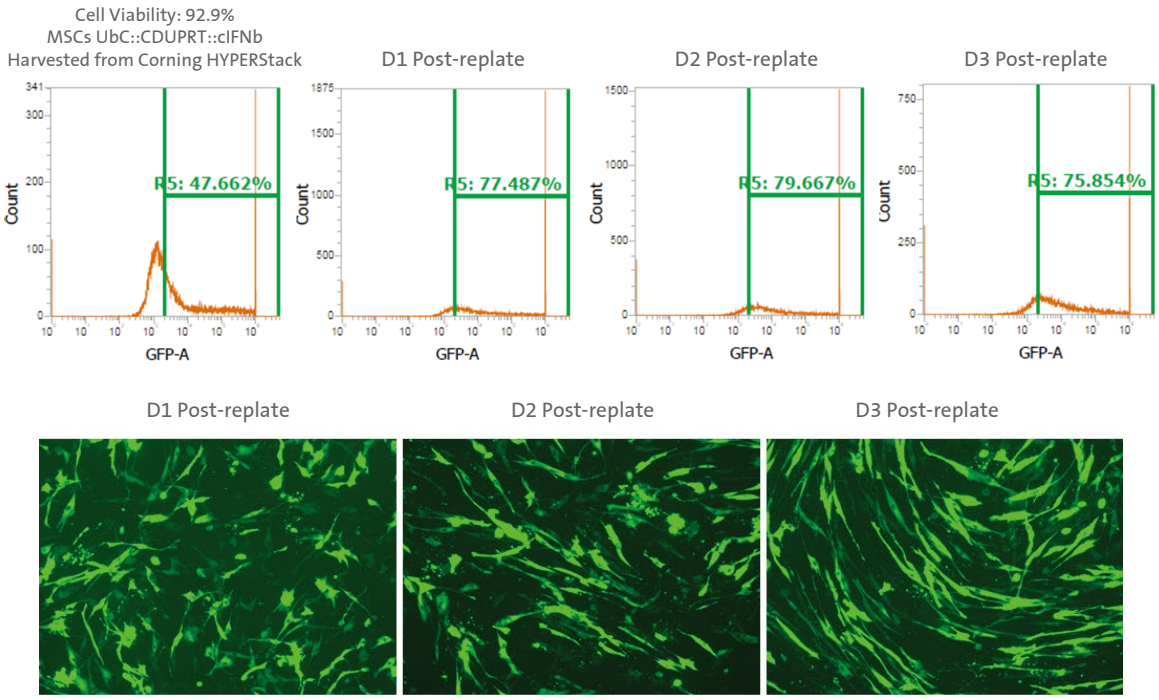

Figure 1. Transgene expression of transfected adipose-derived MSCs expanded in and harvested from Corning HYPERStack 12-layer cell culture vessels.

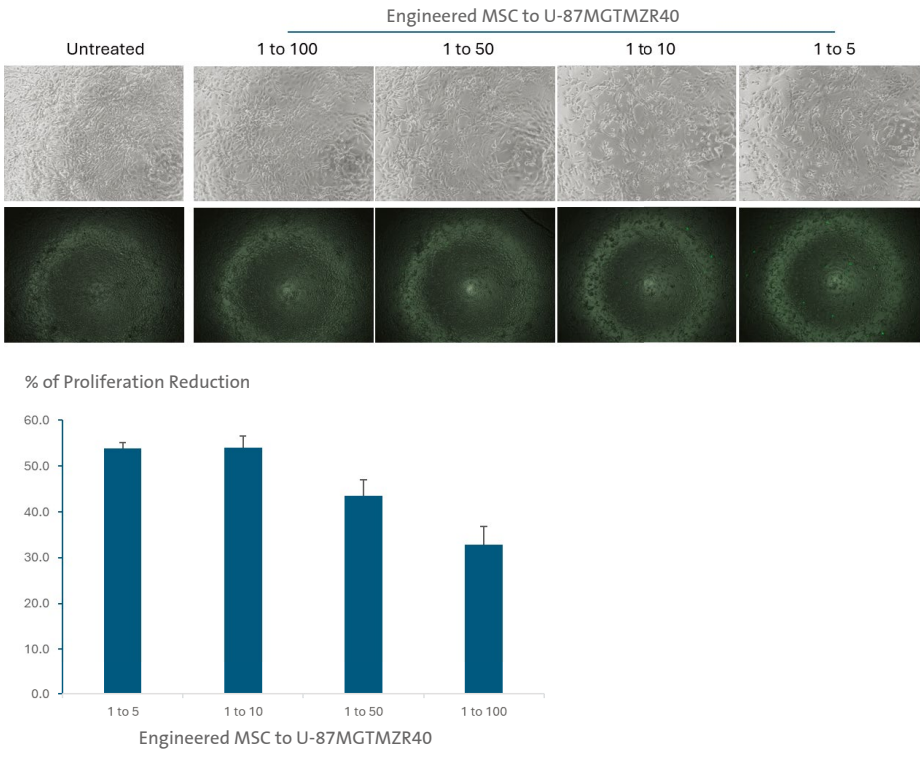

Figure 2. Engineered MSCs produced from a Corning HYPERStack 12-layer cell culture vessel effectively reduce proliferation of drug-resistant glioblastoma cancer cells.

Conclusions

- Within 8 days, both the Corning® CellSTACK® and HYPERStack® systems supported a 100-fold expansion of anti-cancer engineered MSCs—from 2.5 million cryopreserved cells to 270 million using a 2-layer CellSTACK and a 12-layer HYPERStack vessel. This yield could potentially increase to a 300-fold expansion with scale-up to a HYPERStack 36-layer vessel.
- Engineered MSCs consistently maintain transgene expression and anti-cancer potency, as consistently observed across both small-scale and larger culture platforms.

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