Adipose-derived Human Mesenchymal Cell Production in Corning® HYPERStack® 36-layer Cell Culture Vessels

Application Note

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Introduction

Mesenchymal stem cells (MSCs) are multipotent cells that have been generating substantial interest for cellular therapy and regenerative medicine applications for many years. Since Friedenstein, et al.[1] discovered that bone marrow-derived multipotent cells had the ability to differentiate into mesodermal cell types (i.e., adipocytes, osteocytes and chondrocytes)[2] scientists worldwide have been trying to unlock their therapeutic potential. Additionally, they are known to secrete trophic factors (i.e., C-C Ligands, Interleukins, etc.)[3] that can play an important role in immunoregulation[2].

MSCs can be isolated from a variety of tissue sources such as bone marrow, perinatal tissue, subcutaneous adipose tissue, and peripheral blood[4]. However, adipose-derived tissue offers several advantages compared to other source materials; harvesting adipose tissue is less invasive compared to harvesting bone marrow, adipose tissue is more plentiful in the body, and its' stem cell yield is higher[5] as compared to bone marrow.

Here, we demonstrate the utility of the Corning HYPERStack 36-layer cell culture vessel as a tool to scale up and meet the growing demand of adipose-derived MSCs. Our results show that when seeded at 3,000 cells/cm^2 for four days, a single HYPERStack 36-layer vessel yields over 540 million human adipose-derived MSCs that exhibit high viability and express appropriate markers for MSC identity.

Materials and Methods

Single vials of Human adipose-derived MSCs (Lonza PT-5006) were thawed into T-175 flasks (Corning 431080) containing StemMACS™ MSC Expansion Media Kit (Miltenyi Biotec 130-104-182). After 3 to 4 days, upon achieving 90% confluence, cells were harvested with 5 mL TrypLE™ Express Enzyme (Thermo Fisher 12604021) at 37°C for 10 to 15 minutes. Post-harvest, cells were centrifuged at 200 x g for 10 minutes and enumerated using a NucleoCounter® NC-200™ and Via1-Cassette™ (Chemometec). Cells were seeded at a density of 3 x 10^3 cells/cm^2 in a Corning CellBIND® CellSTACK® 1-chamber vessel (Corning 3330) for 4 days. Cells were harvested as previously described and re-plated into two Corning CellBIND CellSTACK 2-chamber vessels (Corning 3310) at 3 x 10^3 cells/cm^2. After four days of culturing at 37°C, cells were harvested and seeded into a Corning HYPERStack 36-layer vessel (Corning 10036) that had been placed into a 37°C CO_2 incubator, to pre-warm, for 24 hours prior to seeding. Following 4 days of culture, cells were harvested and assessed for yield and viability as previously described. To confirm MSC identity, approximately 1 x 10^7 cells were stained with a human MSC analysis kit (BD 562245) (which stains for CD105, CD73, CD90, CD45, CD34, CD11b, CD19, and HLA-DR) per vendor protocol and assessed via flow cytometry with the MACSQuant® Analyzer 10 (Miltenyi Biotec).

Results and Discussion

Figure 1 shows MSC densities ranging from 2.7 x 10^4 to 3.2 x 10^4 cells/cm^2 after 4 days of culture. The average of all three studies resulted in a total MSC yield of over 5.44 x 10^8 cells per HYPERStack 36-layer vessel. For MSCs to be utilized for therapeutic applications, it is essential to recover cells that have good viability and express appropriate markers[6]. Experimental data shows the average MSC viability from HYPERStack 36-layer vessels is greater than 80% (Figure 2).

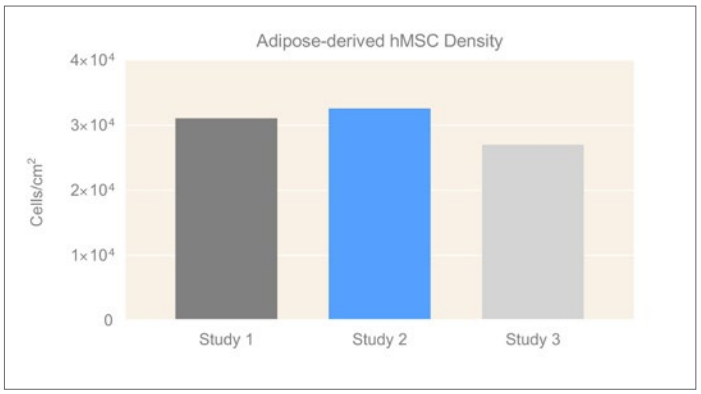

Figure 1. Human MSC recovery. Human adipose-derived MSC recovery from 3 Corning HYPERStack 36-layer vessels.

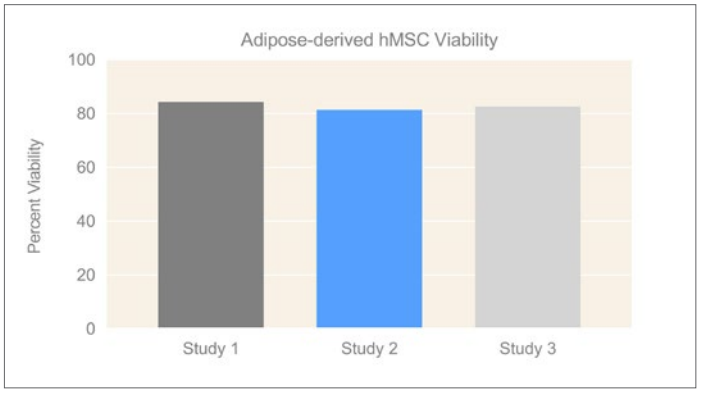

Figure 2. High MSC viability. Adipose-derived MSC viability from 3 Corning HYPERStack 36-layer vessels.

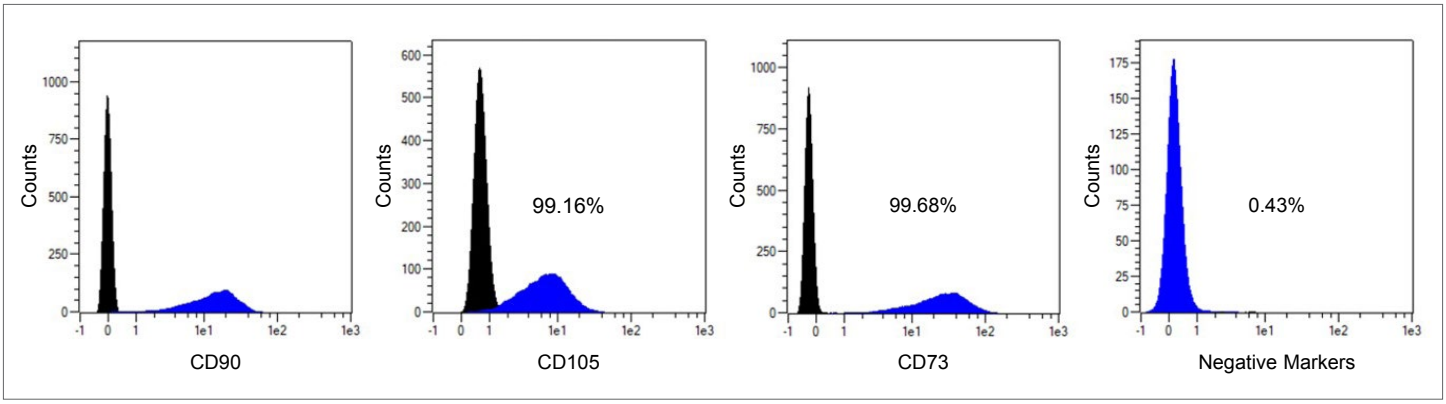

Figure 3. Markers of MSC identity. Representative MSC marker expression from one study. Sample (blue) compared to isotype control (black). Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

The International Society for Cellular Gene Therapy (ISCT) has defined the minimal criteria for hMSC quality as expressing >95% of CD105, CD73, and CD90 and lack of expression (<2%) of typical hematopoietic markers CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules[7]. Figure 3 demonstrates MSCs recovered from HYPERStack 36-layer vessels have greater than 99% expression of CD90, CD105 and CD73 while expressing less than a half a percent of hematopoietic lineage markers (CD45, CD34, CD11b, CD19, and HLA-DR).

Conclusions

With more MSC therapies entering clinical trials, the demand for scale up tools will increase. Corning® HYPERStack® 36-layer cell culture vessels offer a 2D closed system solution for scaling up large quantities of highly viable adipose-derived MSCs expressing the ISCT defined criteria for hMSC quality to meet those growing demands.

References

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