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

Application Note

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Hilary Sherman and Ann Ferrie
Corning Incorporated, Life Sciences
Kennebunk, ME USA

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).

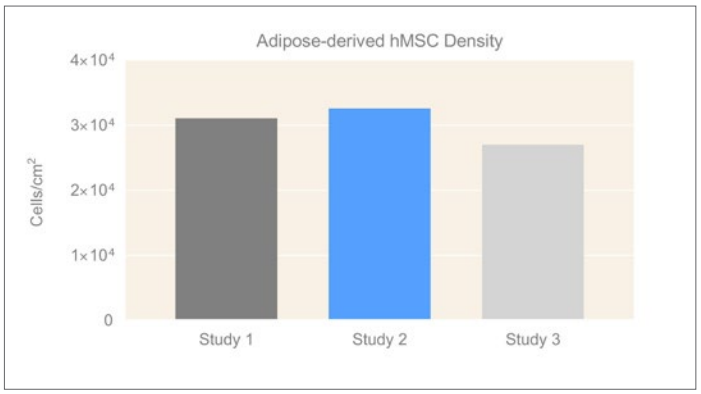

Figure 1. Human MSC recovery. Human adipose-derived MSC recovery from 3 Corning HYPERStack 36-layer vessels.

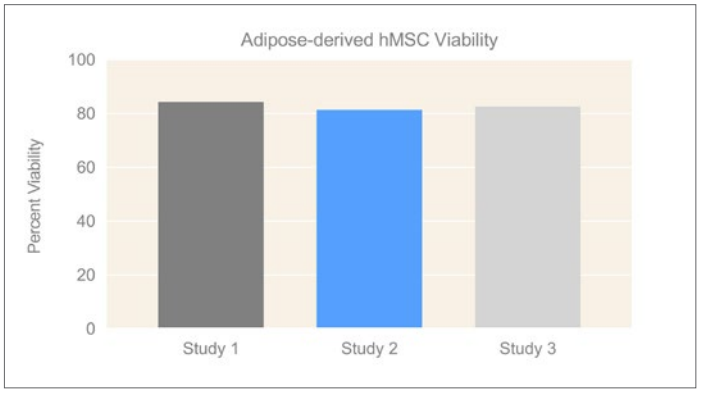

Figure 2. High MSC viability. Adipose-derived MSC viability from 3 Corning HYPERStack 36-layer vessels.

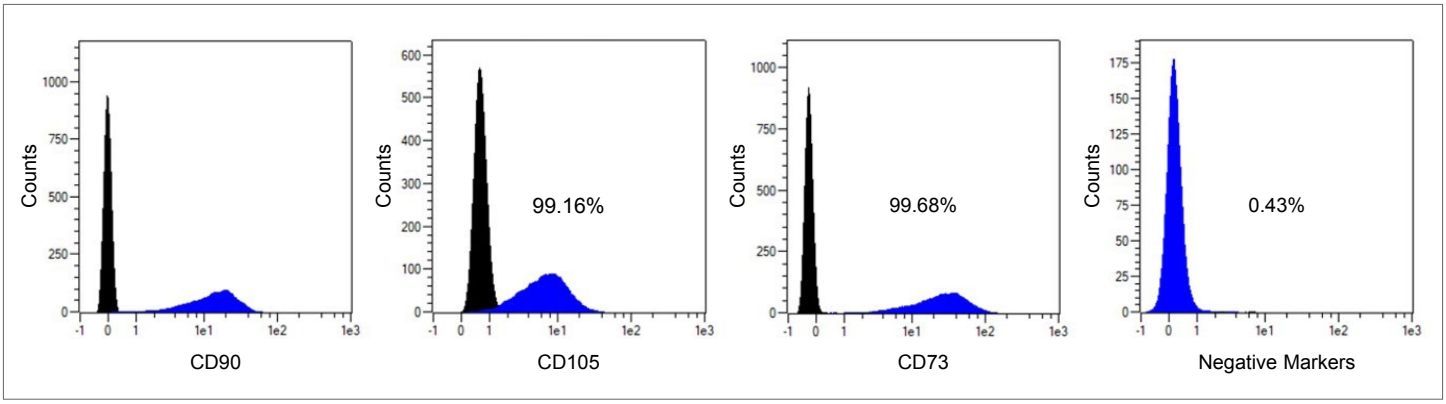

Figure 3. Markers of MSC identity. Representative MSC marker expression from one study. Sample (blue) compared to isotype control (black). Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

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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t 800.492.1110
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EUROPE
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France
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t 0800 376 8660

All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400