Expansion of Human Bone Marrow-derived MSCs with Corning® MSCulture Max™-XF Media and Corning HYPERStack® Cell Culture Vessels

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Application Note

Hilary Sherman and John Shyu
Corning Incorporated, Life Sciences
Kennebunk, ME USA

Introduction

Mesenchymal stem/stromal cells (MSCs) are multipotent cells that have recently generated significant interest for cellular therapy applications. MSCs have the potential to differentiate into other mesenchymal tissue lineages such as adipocytes, osteocytes, and chondrocytes.[1] Additionally, they are known to secrete trophic factors that can play an important role in immunoregulation.[1] Although MSCs can be isolated from different tissue sources, bone marrow-derived MSCs are commonly studied due to their ease of access and achievable therapeutic dosage (2×10^6 cells/kb of body weight).[2,3] Here, we demonstrate the utility of the Corning HYPERStack 36-layer cell culture vessel in conjunction with Corning MSCulture Max™-XF media as a means to meet the growing demand for expanding bone marrow-derived MSCs to relevant scale for clinical application workflows. The utilization of gas permeable film technology provided in the spatial footprint of a traditional stacked cell culture vessel enables Corning HYPERStack cell culture vessels to provide up to 5X the surface area in the same spatial footprint for expansion of adherent cell types, such as MSCs. Our results show that over 870 million human bone marrow-derived MSCs can be obtained from a single HYPERStack 36-layer cell culture vessel when paired with Corning MSCulture Max-XF media. Furthermore, harvested cells demonstrated high viability and expressed characteristic surface markers of human bone marrow-derived MSC identity.

Materials and Methods

Human bone marrow-derived MSCs (RoosterBio MSC-1M-5XF) were thawed into T-175 flasks (Corning 431080) containing Corning MSCulture Max-XF media, which was prepared by combining MSCulture Max basal medium (Corning 42-010-CV) with MSCulture Max-XF supplement (Corning 42-100-CR), per recommended protocol. Upon achieving 90% confluence, cells were harvested with TrypLE™ Express Enzyme (ThermoFisher 12604021) and centrifuged at 200 x g for 10 minutes. Cells were re-plated in Falcon® 875 cm^2 Multi-Flasks (Corning 353144) at a density of 3×10^3 cells/cm^2. After five days of culturing in a humidified, 5% CO_2 incubator at 37°C, cells were harvested as previously described and seeded into pre-warmed HYPERStack 36-layer cell culture vessels (Corning 20036) at 3×10^3 cells/cm^2. It is recommended to pre-warm the HYPERStack 36-layer cell culture vessel at 37°C to prevent any temperature gradients during the seeding process. Human bone marrow-derived MSCs were expanded in the HYPERStack cell culture vessels for five days, then Cells were harvested and assessed for yield and viability.

The expansion from cryogenic vial thaw through HYPERStack 36-layer cell culture vessel expansion was repeated three independent times. To confirm MSC identity, approximately 1×10^7 cells were stained (BD Biosciences 562245) per vendor protocol and CD surface antigens assessed via flow cytometry.

Results and Discussion

Human bone marrow-derived MSC densities ranging from 4.4×10^4 to 5.2×10^4 cells/cm^2 were achieved after 5 days of culture in HYPERStack 36-layer cell culture vessels utilizing Corning MSCulture Max-XF media (Figure 1). The average of all three studies resulted in a total MSC yield of more than 8.7×10^8 cells per HYPERStack 36-layer cell culture vessel. For therapeutic application workflows, it is essential to recover MSCs that have high viability and express appropriate surface markers.[4] In these studies, MSCs collected from HYPERStack 36-layer cell culture vessels showed greater than 90% average viability (Figure 1). The International Society for Cellular Gene Therapy (ISCT) has defined the minimal criteria for human bone marrow-derived MSC 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.[4] Human bone marrow-derived MSCs recovered from HYPERStack 36-layer cell culture vessels in conjunction with Corning MSCulture Max-XF media were characterized via flow cytometry, and displayed greater than 99% expression of CD90, CD105, and CD73 while expressing less than a half a percent of negative markers (CD45, CD34, CD11b, CD19, and HLA-DR).

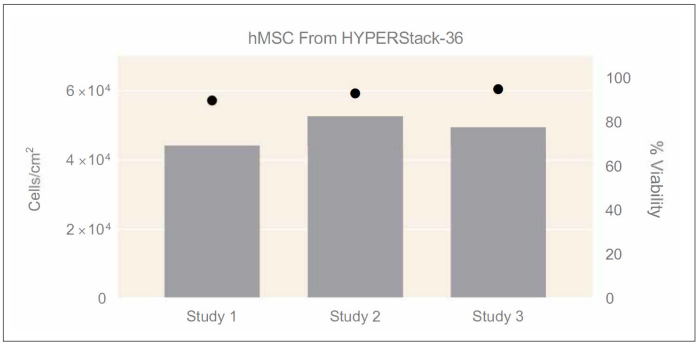

Figure 1. Human bone marrow-derived MSC yields and viability from Corning HYPERStack 36-layer cell culture vessels. Bars are cell density and dots are viability.

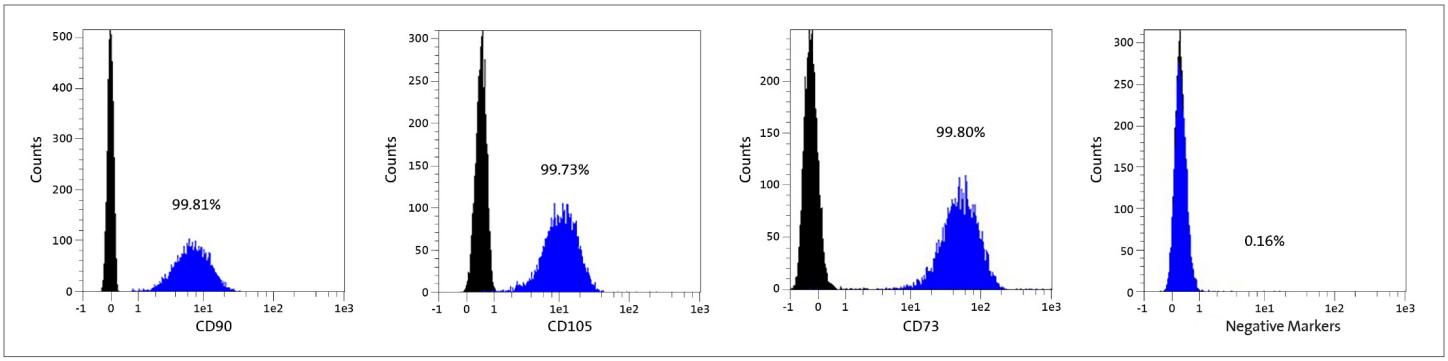

Figure 2. Surface marker characterization of human bone marrow-derived MSCs expanded in Corning HYPERStack 36-layer cell culture vessels. Representative marker expression from one study. Sample (blue) compared to isotype control (black). Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

Conclusions

The growing number of clinical trials involving stem cells used for therapeutic applications has led to an increase in demand for tools and technologies that enable efficient scale-up of stem cells, without compromising cell viability and cell health. Here we demonstrated that the Corning® HYPERStack® 36-layer cell culture vessels in conjunction with Corning MSCulture Max™-XF media offer an effective method for expanding large quantities of human bone marrow-derived MSCs that maintain high viability and appropriate surface marker characterization.

References

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