Expansion of Human Umbilical Cord-derived MSCs using Corning® MSCulture Max™-XF Media and Corning HYPERStack® 36-layer Cell Culture Vessels

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Application Note

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Introduction

Mesenchymal stem/stromal cells (MSCs), which are multipotent stromal cells, have recently attracted significant interest for their possible use in regenerative medicine applications. As a multipotent cell, MSCs have the potential to differentiate into other 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 a variety of tissue sources, human umbilical (HU) cord-derived MSCs offer several advantages.[2] HU MSCs are collected from discarded tissue after birth which means there is no additional procedure required for collection.[2] Additionally, they have been shown to grow faster and can be maintained longer *in vitro*.[2] In order to maintain and expand MSCs *in vitro* it is essential to provide the appropriate cell culture environment. Corning MSCulture Max-XF media in combination with Corning CellBIND® surface treated Corning HYPERStack cell culture vessels are an ideal solution. Our results show that over 1 billion human umbilical cord-derived MSCs can be obtained from a single HYPERStack 36-layer vessel. Furthermore, harvested cells have high viability and express markers often used in MSC identity.

Materials and Methods

Human umbilical cord-derived MSCs (RoosterBio hUC-1M-XF) were thawed into T-175 flasks (Corning 431080) containing Corning MSCulture Max-XF media (Corning 42-100-KIT). Upon achieving 90% confluence, cells were harvested with TrypLE™ Express Enzyme (Thermo Fisher 12604021) and centrifuged at 200 x g for 10 minutes. Cells were re-plated in Corning HYPERFlask® M cell culture vessels (Corning 10030) at a density of 3 x 10^3 cells/cm^2. After five days of culturing at 37°C, cells were harvested as previously described and seeded into pre-warmed HYPERStack 36-layer vessels (Corning 10036)* at 3 x 10^3 cells/cm^2. It is recommended to pre-warm the HYPERStack 36-layer vessel at 37°C to prevent any temperature gradients during the seeding process. Following five days of culture, cells were harvested and assessed for yield and viability. To confirm MSC identity, approximately 1 x 10^7 cells were stained (BD 562245) per vendor's protocol and assessed via flow cytometry.

Results and Discussion

Figure 1 shows MSC densities ranging from 6.1 x 10^4 to 8.0 x 10^4 cells/cm^2 after 5 days of culture. The average of all three studies results in a total MSC yield of over 1.24 x 10^9 cells per HYPERStack 36-layer vessel. For MSCs to have therapeutic applications, it is essential to recover cells that have high viability and express appropriate markers[3]. Our data shows greater than 85% average MSC viability from HYPERStack 36-layer vessels (Figure 2). 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[4]. Figure 3 demonstrates

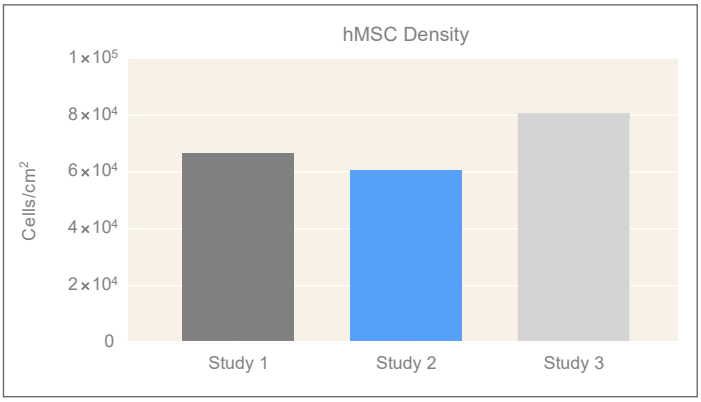

Figure 1. Human MSC density. Human umbilical cord-derived MSC recovery from 3 Corning HYPERStack-36 cell culture vessels.

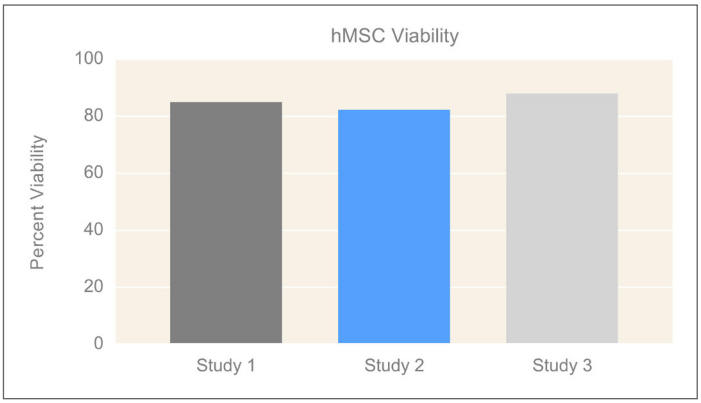

Figure 2. High MSC viability. Human umbilical cord-derived MSC viability from 3 Corning HYPERStack-36 cell culture vessels.

*After the study was completed, Corning introduced design enhancements for the HYPERStack vessel (Corning 20036, previously Corning 10036), which improved performance but did not affect its use for MSC production as described in this application note.

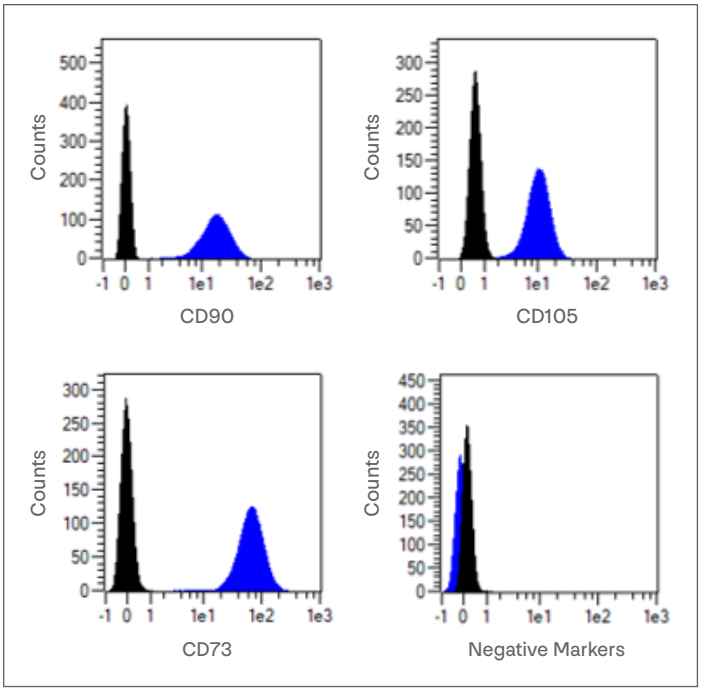

Figure 3. Appropriate markers of MSC identity. Representative MSC marker expressions from one study. Sample in blue compared to isotype control in black. Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

MSCs recovered from HYPERStack 36-layer vessels have greater than 99% expression of CD90, CD105, and CD73 while expressing less than a half of one percent (<0.5%) of negative markers (CD45, CD34, CD11b, CD19, and HLA-DR).

Conclusions

As more clinical trials are evaluating MSC-based therapies, the demand for more pertinent adherent scale-up tools is likely to increase. Corning® HYPERStack® 36-layer cell culture vessels offer a closed system solution for scaling up large quantities of quality, human umbilical cord-derived MSCs.

Additionally, MSCs cultured with Corning MSCulture Max™-XF media in the HYPERStack 36-layer vessel expressed high percentages of CD90, CD105 and CD73, characteristic of MSC quality. The ability to grow large quantities of human umbilical cord-derived MSCs with high viability and relevant expression markers will further expand their usage in therapeutic applications.

References

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