Human Umbilical Cord-derived Mesenchymal Stem Cell Production in Corning® HYPERStack® 36-layer Cell Culture Vessels

Application Note

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Introduction

Mesenchymal stem 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]. 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 RoosterNourish™-MSC-XF (RoosterBio KT-016) per vendor's recommended protocol. 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 Corning HYPERStack 36-layer vessels (Corning 10036) at 3 x 10^3 cells/cm^2. It is recommended to pre-warm the Corning 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 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).

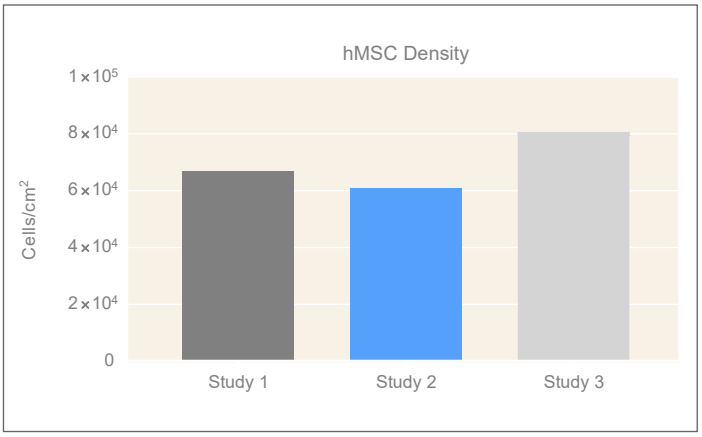

Figure 1. Human MSC recovery. Human umbilical cord-derived MSC recovery from 3 Corning HYPERStack-36 cell culture vessels.

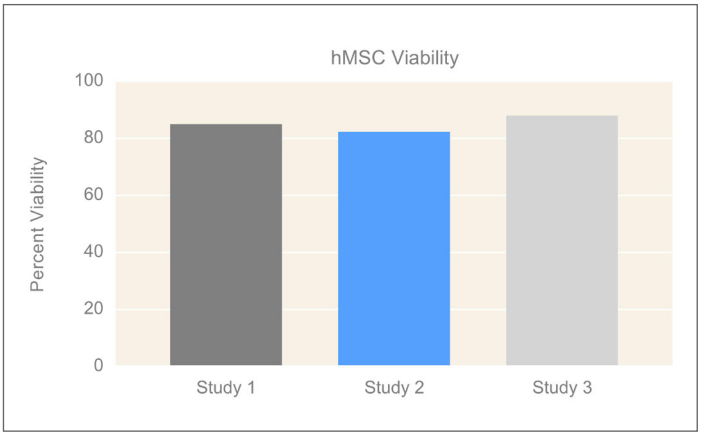

Figure 2. High MSC viability. Human umbilical cord-derived MSC viability from 3 Corning HYPERStack-36 cell culture vessels.

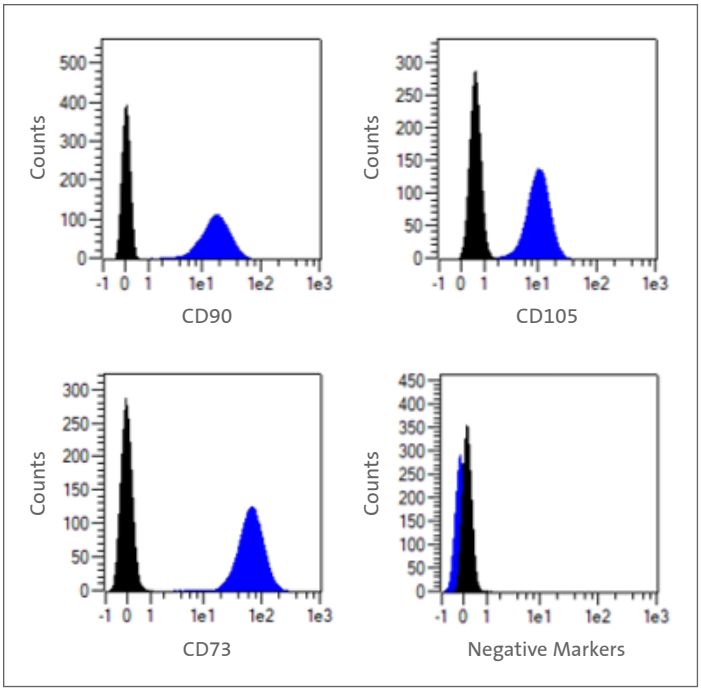

Figure 3. Appropriate markers of MSC identity. Representative MSC marker expression from one study. Sample in blue compared to isotype control in black. Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

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. Most importantly, the MSCs expanded 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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