

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

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## Application Note

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

### 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<sup>1</sup>. Additionally, they are known to secrete trophic factors that can play an important role in immunoregulation<sup>1</sup>. Although, MSCs can be isolated from a variety of tissue sources, human umbilical (HU) cord-derived MSCs offer several advantages<sup>2</sup>. HU MSCs are collected from discarded tissue after birth which means there is no additional procedure required for collection<sup>2</sup>. Additionally, they have been shown to grow faster and can be maintained longer *in vitro*<sup>2</sup>. 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.

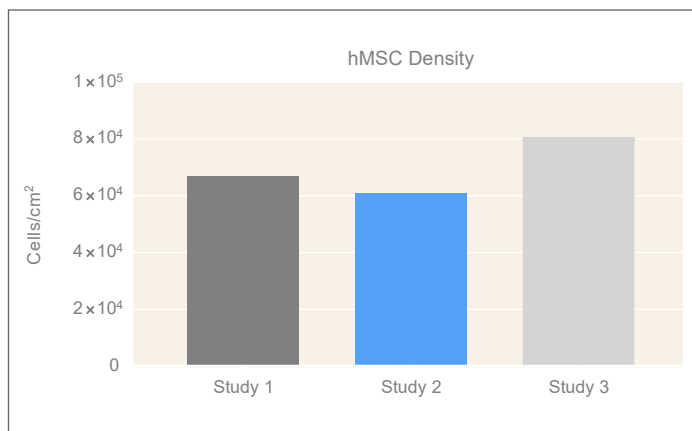
### Materials and Methods

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  $3 \times 10^3$  cells/cm<sup>2</sup>. 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  $3 \times 10^3$  cells/cm<sup>2</sup>. 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  $1 \times 10^7$  cells were stained (BD 562245) per vendor's protocol and assessed via flow cytometry.

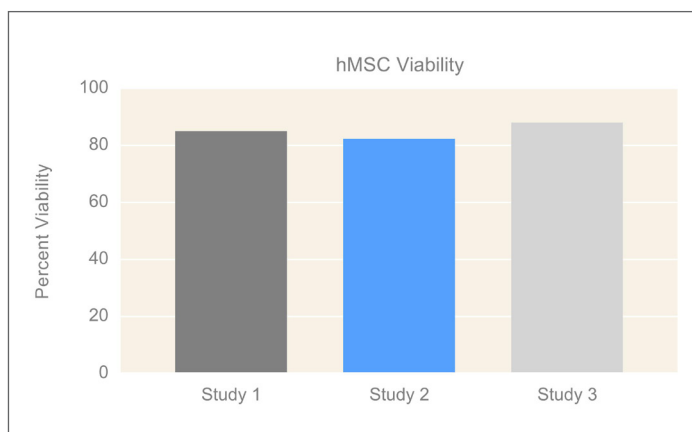
### Results and Discussion

Figure 1 shows MSC densities ranging from  $6.1 \times 10^4$  to  $8.0 \times 10^4$  cells/cm<sup>2</sup> after 5 days of culture. The average of all three studies results in a total MSC yield of over  $1.24 \times 10^9$  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<sup>3</sup>. Our data shows greater than 85% average

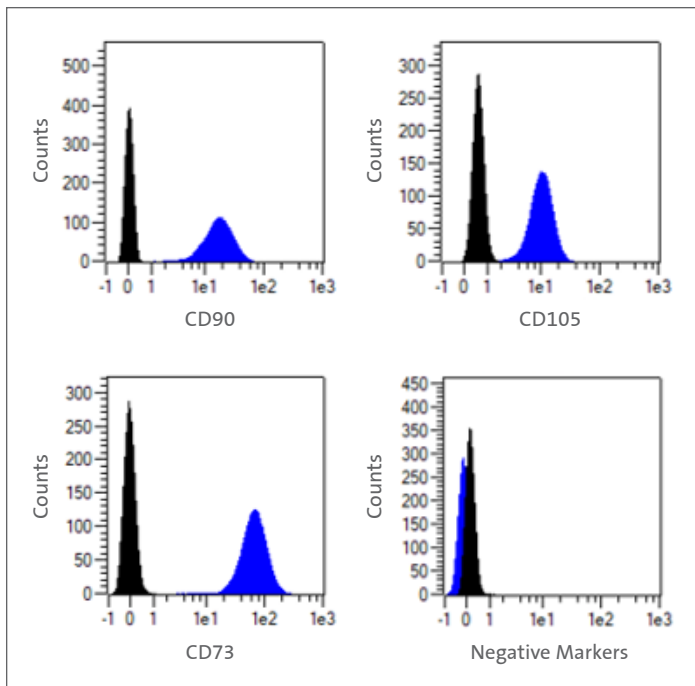
MSC viability from HYPERStack 36-layer vessels (Figure 2). The International Society for Cellular Gene Therapy (ISCT) has defined the minimal criteria for hMSC quality as expressing >95% of CD105, CD73, and CD90 and lack of expression (<2%) of typical hematopoietic markers CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules<sup>4</sup>. 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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4. Robb KP, et al. Mesenchymal stromal cell therapy: progress in manufacturing and assessments of potency. *Cytotherapy* 21.3:289-306 (2019).

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