

Corning® MSCulture Max™-XF Media Outperforms Serum-free, Serum-containing, Xeno-free, and Animal-free Alternatives

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Application Note

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Introduction

Mesenchymal stem/stromal cells (MSCs) are multipotent cells that have the potential to differentiate into other mesenchymal tissue lineages such as adipocytes, osteocytes, and chondrocytes¹. Researchers have exploited this multipotency to treat patients for a variety of diseases and depending on the disease, dosing could consist of millions to hundreds of millions of MSCs per patient². Because of these quantities, it is essential to be able to expand the cells as much as possible while maintaining cell quality. Additionally, reducing labor and reagent costs can provide important cost savings, making MSC therapy a more practical treatment option. Corning MSCulture Max-XF media is a xeno-free solution for MSC culture that allows for robust expansion without the need for a surface coating or media exchanges during culture, as required by many other commercially available media formulations. Here, we compare MSC expansion and cell quality from several media options including serum-free, serum-containing, xeno-free, and animal-free formulations vs. MSCulture Max-XF media.

Materials and Methods

Serum-free and animal-free media were prepared per vendor recommended protocols which in some cases included supplementation with L-Glutamine (Corning 25-005-CI). Corning MSCulture Max-XF media was prepared by combining MSCulture Max basal medium (Corning 42-010-CV) with MSCulture Max-XF supplement (Corning 42-100-CR), per recommended protocol. Serum-containing medium was Corning MEM (Minimum Essential Medium) Alpha medium (Corning 10-022-CV) containing 10% FBS (Corning 35-010-CV) and 2 mM L-Glutamine. Xeno-free medium was prepared by supplementing basal medium with 5% human platelet lysate (HPL; Sartorius PLTGOLD27R). All media was stored and used in accordance with vendor recommendations and shelf lives. Cryopreserved human MSCs (MilliporeSigma SCC034) were thawed directly into 3 mL of complete serum-containing medium and enumerated using a NucleoCounter® NC-200™ and Via1-Cassette™

(Chemometec). Cells were then aliquoted into each medium at a concentration of 1.58×10^4 cells/mL. All 6 wells of a Corning CellBIND® 6-well clear multiwell plate (Corning 3335) were used for each media formulation and seeded at 5,000 cells/cm² using 3 mL of medium per well. Two of the commercially available media required coatings to aid in cell attachment, so the Corning CellBIND 6-well clear multiwell plates were coated per vendor recommended protocols prior to initiating cultures. Cells were cultured for 5 days with medium exchanges performed as recommended by the manufacturer. On the last day of culture, cells were imaged and harvested using Accutase® cell detachment solution (Corning 25-058-CI) and 3 wells per condition were counted. After counting, all 6 wells of each medium were pooled for multipotency analysis via flow cytometry. Cells were stained using BD Stemflow™ Human MSC Analysis Kit (BD Biosciences 562245) following the manufacturer's protocol and assessed via flow cytometry with the MACSQuant® Analyzer 10 (Miltenyi Biotec). The study was repeated 3 independent times for a total of 9 replicates per media.

Results and Discussion

MSC morphology has been shown to correlate with expansion capacity and differentiation potential³. Typically, smaller, less spindle-like morphologies are generally associated with higher proliferation and greater differentiation potential while more cuboidal morphologies can see decreased expansion and tend to be more downregulated for one of the commonly used markers of multipotency (CD73)³. Figure 1 shows representative images of MSC morphologies and confluence after 5 days of growth in all five media evaluated. MSCs cultured in Corning MSCulture Max-XF media were the only culture to reach confluence by day 5. Figure 2 shows MSC densities of harvested cells from each medium tested, and the results show statistically higher cell densities recovered from MSCulture Max-XF media compared to all other media while also maintaining high viability (Figure 3). Finally, the International Society for Cellular Gene Therapy (ISCT) has defined the minimal

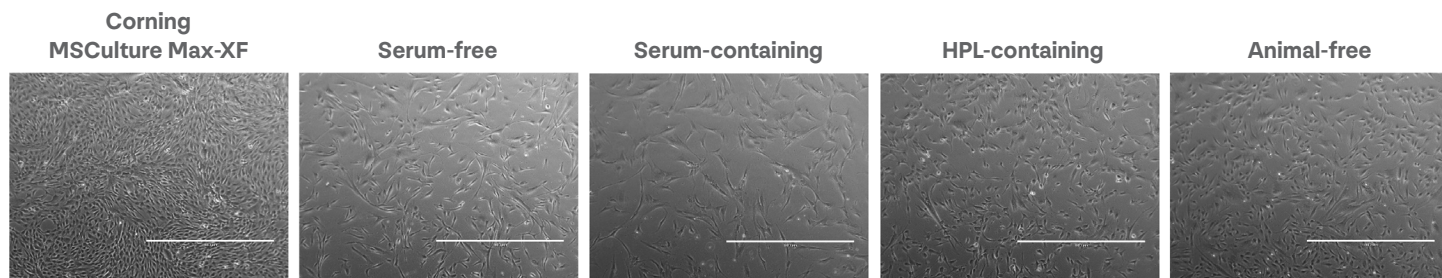


Figure 1. Human MSC Morphology. Representative brightfield images of MSC morphology after 5 days when cultured in various media formulations. Scale is 1000 µm.

criteria for hMSC quality as expressing >95% of CD105, CD73, and CD90 and lack of expression of typical hematopoietic markers CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules*. Figure 4 shows representative marker expression of MSCs from all media types showing that MSCs cultured in MSCulture Max-XF media demonstrate high expression of positive markers and a lack expression of negative markers.

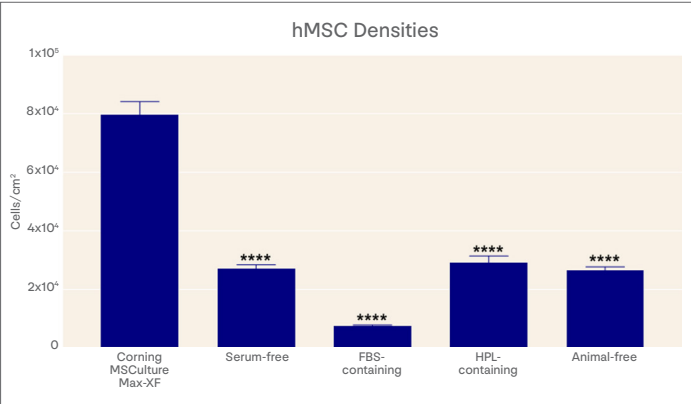


Figure 2. Human MSC Densities. MSC densities harvested from multiple media formulations. Data is the average from 3 independent studies shown with standard deviation (n=9). Asterisks indicate statistical difference compared to Corning MSCulture Max-XF media, ****= $p < 0.0001$.

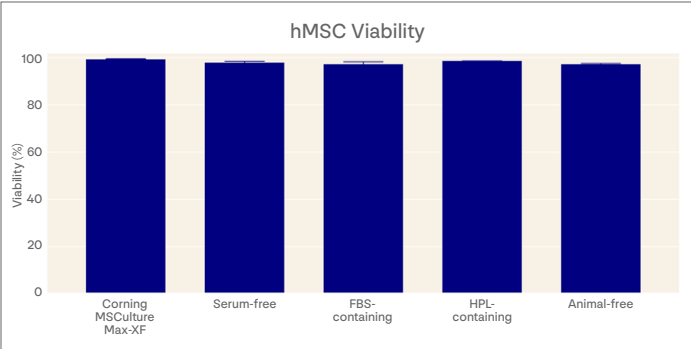


Figure 3. High MSC Viability. MSC viability from multiple media formulations. Data is the average from 3 independent studies shown with standard deviation.

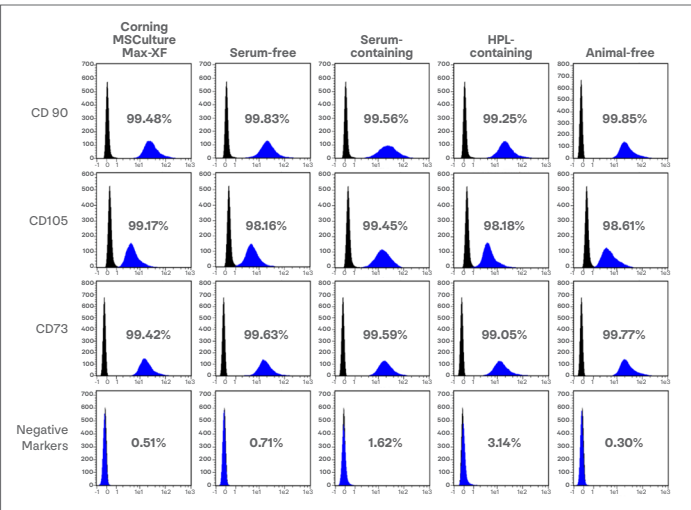


Figure 4. Appropriate Markers of MSC Identity. Representative MSC marker expression from one study. Sample in blue compared to isotype control in black. Negative markers are a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR.

Conclusions

Due to the sensitivity of MSCs and the quantities required for applications, having the right growth environment to maximize expansion and ensure quality is essential. Many commercially available MSC media require specialized coatings for cell attachment or frequent media exchanges during culture to ensure success. These additional steps add complexity, cost, and increase the risk of contamination to an already challenging process. Corning® MSCulture Max™-XF media supports robust expansion of high quality MSC using standard cell culture ware without a coating or media exchanges.

References

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