

New Basal Medium to Support hPL-Supplemented Expansion of MSCs

Using PRIME-XV MSC Basal XSFM

INTRODUCTION

Mesenchymal stromal/stem cells (MSCs) have shown great promise in the development of cellular therapies due to their immunomodulatory/suppressive properties and capability to differentiate into lineages suitable to support regenerative medicine applications. Despite these recognizable clinical outcomes, MSCs still struggle to reach the same levels of approval by regulatory bodies as other cellular therapies like T cells. This is due to a combination of challenges in understanding the mechanism of action behind the clinical outcomes and the challenges in controlling the manufacturing process at scale. Many factors are involved in developing a manufacturing process that can be translated for clinical applications, one major factor being critical raw materials like cell culture media.

Classical media supplements like fetal bovine serum (FBS) are still widely used in early stages of development for MSC-based therapies. However, the use of a xeno-derived reagent, such as FBS, carries several challenges, from the risk of adventitious agent carryover to the potential impact on the reproducibility of the manufacturing processes. In this regard, human platelet lysate (hPL) is a superior alternative to FBS, as the controlled lysis of a large number of pooled platelets leads to a remarkably robust end product, which provides developers with a consistent and cost-effective compromise to animal-derived products.

Control over the outcome of manufacturing processes may be further enhanced by the optimization of growth conditions. Alpha minimum essential medium (α MEM) is a well-tested medium that supports a broad number of cell culture applications, in particular the expansion of MSCs when supplemented with hPL. Nevertheless, a thorough understanding of the biologic behavior and metabolic needs of MSCs has prompted FUJIFILM Biosciences to develop an MSC-specific serum- and xeno-free basal medium, PRIME-XV MSC Basal XSFM, that maximizes the outcome of hPL supplementation in supporting human MSC expansion and further enables process outcome control at scale.

In this application note, we aim to explore how PRIME-XV MSC Basal XSFM supplemented with hPL performs in expanding MSCs from different sources when compared with classical media supplementations and several commercially-available MSC media. We also aim to investigate how expansion of MSCs in PRIME-XV MSC Basal XSFM affects the quality of the cells as assessed by surface markers and differentiation capability.



MATERIALS

Reagents and Equipment		
<ul style="list-style-type: none"> • PRIME-XV MSC Basal XSFM (FUJIFILM Biosciences, Catalog # 91235G) • PRIME-XV MSC Expansion XSFM (FUJIFILM Biosciences, Catalog # 91149) • PRIME-XV Human Fibronectin (FUJIFILM Biosciences, Catalog # 31002) • Alpha MEM Earle's Salts (FUJIFILM Biosciences, Catalog # 9144) • Alpha MEM Earle's Salts w/o Nucleosides (FUJIFILM Biosciences, Catalog # 9142) • PLTGold-GI Clinical Grade Human Platelet Lysate (Mill Creek Life Sciences, Catalog # PLTGold27GMP-GI) • PLTMax Research Grade Human Platelet Lysate (Mill Creek Life Sciences, Catalog # PLTMax27R) 	<ul style="list-style-type: none"> • Invitrogen LIVE/DEAD Fixable Blue Dead Cell Stain (Thermo Fisher Scientific, Catalog # L34962) • ViaStain AOPI Staining Solution (Revity, Catalog # CS2-0106-25ML) • Cellaca MX High-throughput Cell Counter (Revvity) • BD FACSymphony A5 Cell Analyzer (BD Biosciences) 	<ul style="list-style-type: none"> • CD14 (BioLegend, Catalog # 612763) • CD19 (BioLegend, Catalog # 563325) • CD34 (BioLegend, Catalog # 343604) • CD45 (BioLegend, Catalog # 304028) • CD73 (BioLegend, Catalog # 344010) • CD90 (BioLegend, Catalog # 328114) • CD105 (BioLegend, Catalog # 323206) • HLA-DR (BioLegend, Catalog # 307645)

METHODS

MSC Culture

Human MSCs were purchased from commercial- available sources. MSCs were expanded in PRIME-XV MSC Basal XSFM, PRIME-XV MSC Expansion XSFM, Alpha MEM Earle's Salts, Alpha MEM Earle's Salts w/o Nucleosides, or commercially-available MSC media. Basal media were supplemented with PLTGold-GI, PLTMax, or commercially-available FBS. Where specifically required, MSCs were grown on tissue culture- (TC) treated surfaces with fibronectin coating. All media conditions containing hPL (PLTGold-GI or PLTMax) were grown on TC-treated surfaces without any coating. Cultures were maintained by replacing the respective media every 2 to 3 days and the cells were passaged once a confluence of 70% to 85% was achieved.

MSC Characterization

During cell passage, cell suspension was mixed with ViaStain AOPI Staining Solution and counted using the Cellaca MX High-throughput Cell Counter. For surface marker analysis, the cells were stained with antibodies against markers of interest and analyzed via flow cytometry.

MSC Differentiation

For trilineage differentiation, commercially-available differentiation media were used. The resulting cells were stained using Alcian Blue, Alizarin Red S, or Oil Red O and imaged by phase contrast microscopy to assess differentiation.

RESULTS

PRIME-XV MSC Basal XSFM was supplemented with hPL (PLTGold-GI) to assess expansion of human MSCs from 3 different tissue origins: bone marrow (BM), adipose (AD), and umbilical cord (UC). Using 3 different donors per tissue type, PRIME-XV MSC Basal XSFM with 5% hPL supported robust expansion of all tested MSCs (**Figure 1**). After 4 passages, MSCs cultured in MSC Basal XSFM displayed the characteristic MSC surface markers as assessed by flow cytometry, demonstrating that the medium supports the maintenance of MSC phenotype (**Table 1**).

MSC Expansion Across Four Passages (14 Days of Culture)

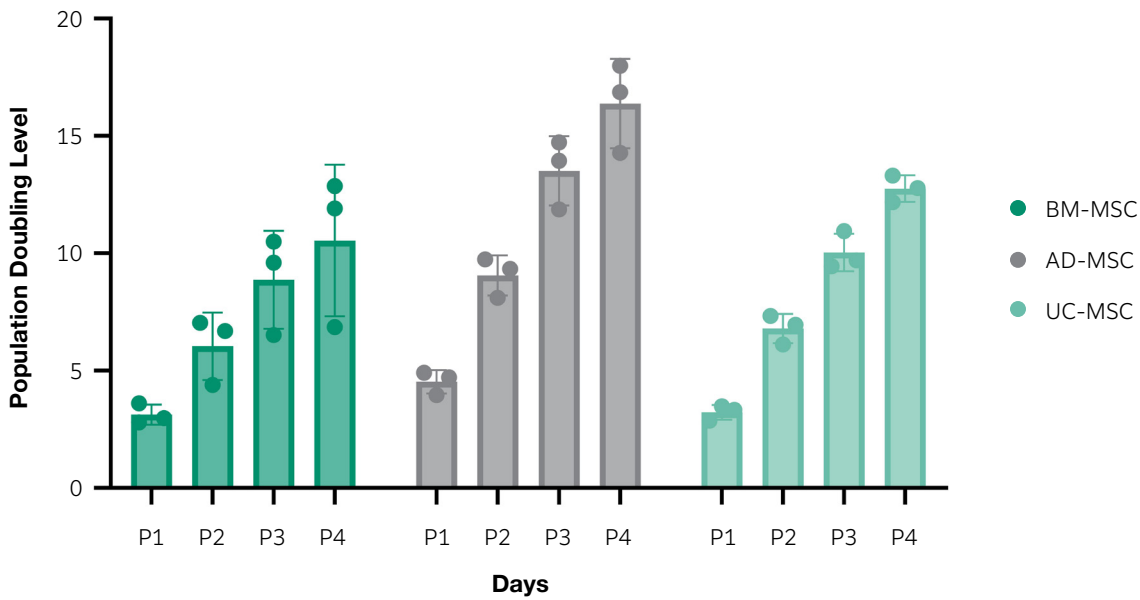


Figure 1. PRIME-XV MSC Basal XSFM supplemented with 5% hPL successfully supported expansion of various sources of hMSCs. Over 4 passages of culture, PRIME-XV MSC Basal XSFM supplemented with 5% hPL (PLTGold-GI, Mill Creek) supports reliable expansion of hMSCs across 3 different tissue origins. Each data point represents the average of a single donor, with a total of 3 donors tested per tissue origin.

Table 1. PRIME-XV MSC Basal XSFM supplemented with 5% hPL (PLTGold-GI, Mill Creek) maintains MSC phenotype based on surface markers.

Sample	CD73	CD90	CD105	CD34	CD45	CD14	CD19	HLA-DR
BM-MSC	100.0%	100.0%	99.1%	0.11%	0.114%	0.021%	0.067%	0.016%
AD-MSC	99.5%	99.8%	99.7%	0.036%	0.089%	0.042%	0.125%	0.003%
UC-MSC	100.0%	100.0%	97.5%	0.056%	0.043%	0.036%	0.043%	0.027%

The surface marker data show the average of 3 different donors for each source of hMSC measured after 4 passages. Positive markers (CD73, CD90, CD105) are maintained, negative markers (CD34, CD45, CD14, CD19, HLA-DR) remained low.

The performance of PRIME-XV MSC Basal XFSM was compared against Alpha MEM Earle's Salts and Alpha MEM Earle's Salts w/o Nucleosides, all supplemented with 5% hPL. After 14 days of MSC expansion, MSC Basal XFSM supplemented with 5% hPL outperformed Alpha MEM Earle's Salts supplemented with 5% hPL by ~2 total population doublings (i.e., ~4-fold greater expansion) (**Figure 2**). Under the same 5% hPL supplementation, MSC Basal XFSM outperformed Alpha MEM Earle's Salts w/o Nucleosides by ~4 total population doublings (i.e., ~16-fold greater expansion).

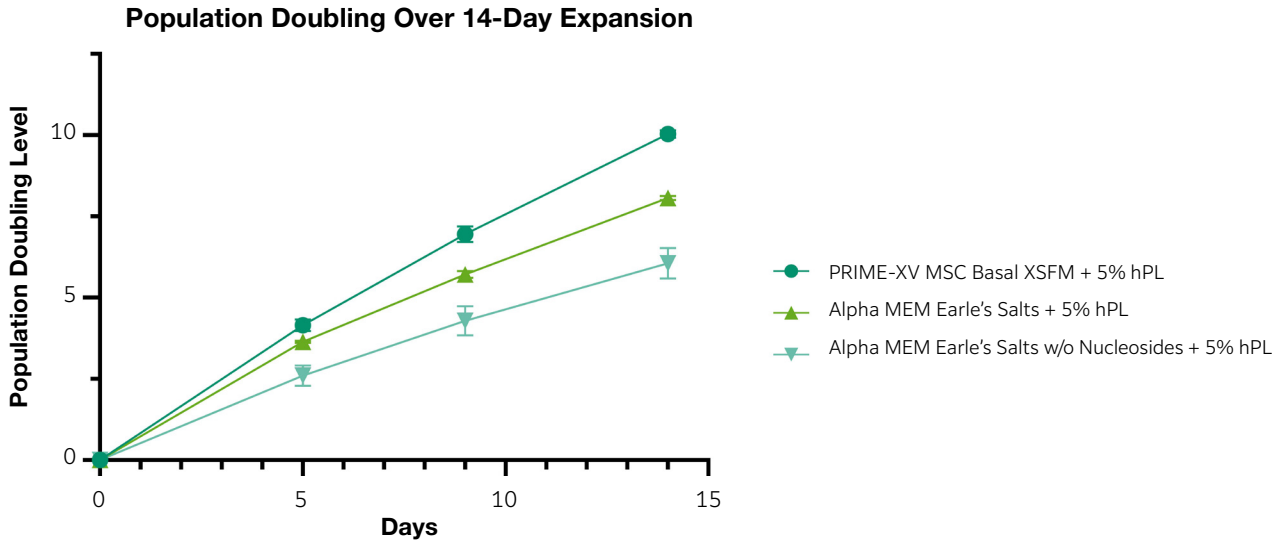


Figure 2. PRIME-XV MSC Basal XFSM supplemented with 5% hPL outperformed Alpha MEM Earle's Salts and Alpha MEM Earle's Salts w/o Nucleosides supplemented with 5% hPL. Data shows a minimum of triplicate measurements from a single donor (BM-MSC). The performance of PRIME-XV MSC Basal XFSM was compared against other commercially-available basal media supplemented with hPL. PRIME-XV MSC Basal XFSM supplemented with 5% hPL also outperformed Alpha MEM Earle's Salts and Alpha MEM Earle's Salts w/o Nucleosides supplemented with 5% hPL (**Figure 3**).

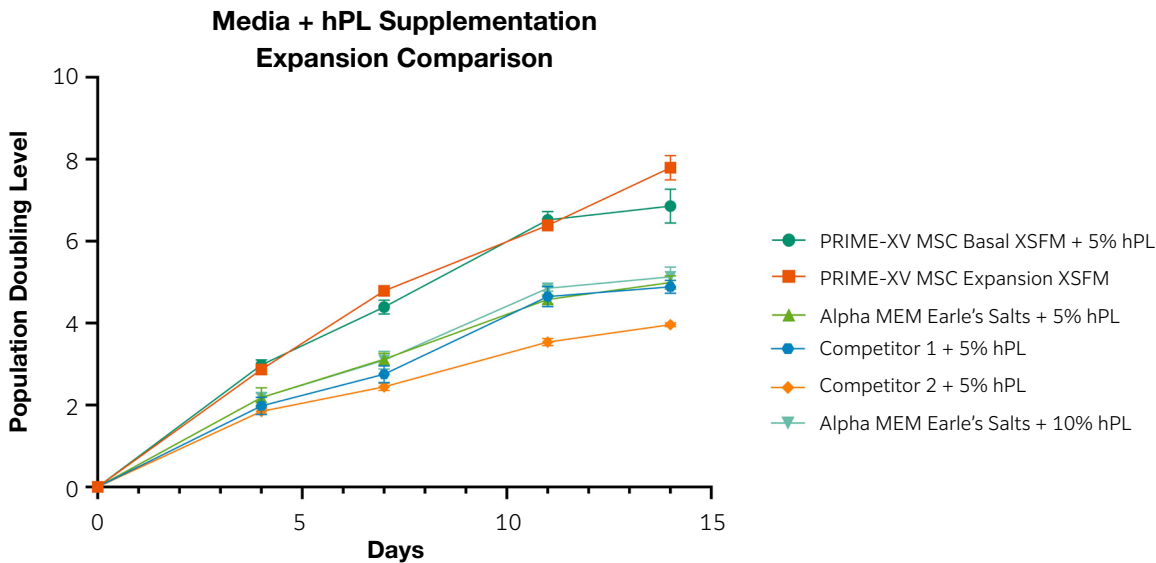


Figure 3. PRIME-XV MSC Basal XFSM supplemented with 5% hPL supported robust expansion of hMSCs. Data shows a minimum of triplicate measurements from a single donor (BM-MSC). In addition to competitor MSC basal media, PRIME-XV MSC Basal XFSM supplemented with 5% hPL was compared against other commercially-available, complete MSC media (without additional hPL supplementation). Over the course of 4 passages, PRIME-XV MSC Basal XFSM with 5% hPL outperformed all tested competitor media. PRIME-XV MSC Basal XFSM with 5% hPL also significantly outperformed Alpha MEM Earle's Salts and Alpha MEM Earle's Salts w/o Nucleosides supplemented with 10% FBS (**Figure 4**).

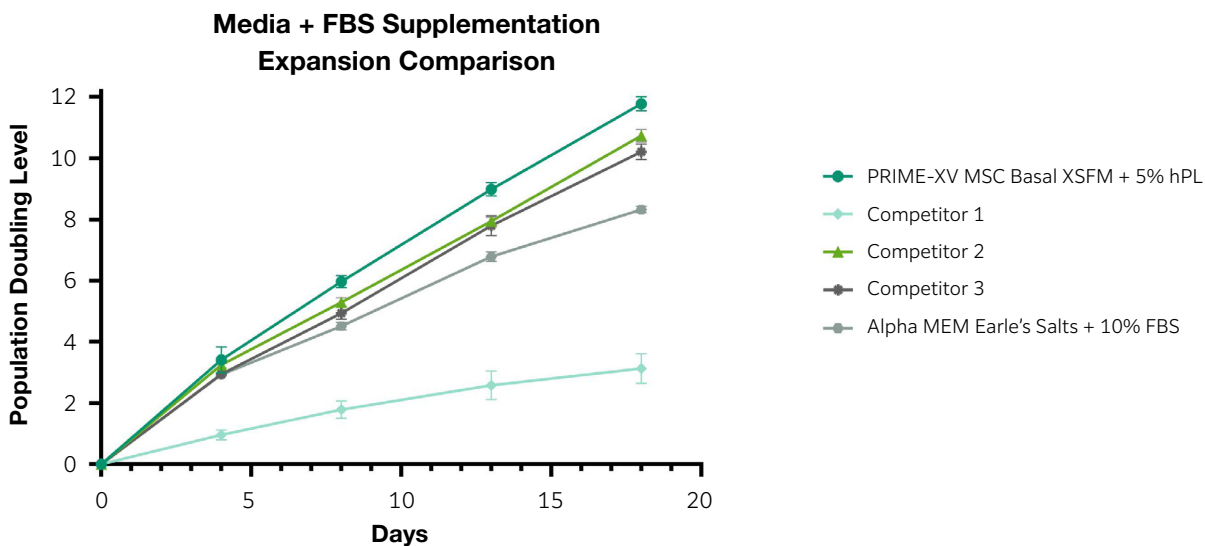


Figure 4. PRIME-XV MSC Basal XFSM supplemented with 5% hPL outperformed competitor MSC media. Data shows a minimum of triplicate measurements from a single donor (BM-MSC) (Table 2).

Table 2. After 4 passages, MSCs cultured in PRIME-XV MSC Basal XFSM and competitor media all displayed the following MSC characteristic surface markers assessed by flow cytometry.

Sample	CD73	CD90	CD105	CD34	CD45	CD14	CD19	HLA-DR
PRIME-XV MSC Basal XFSM + 5% hPL	99.9%	99.9%	99.8%	0.14%	0.21%	0.20%	0.07%	0.03%
Competitor 1	99.9%	100.0%	99.9%	20.4%	16.0%	0.02%	0.10%	25.4%
Competitor 2	100.0%	100.0%	99.8%	0.07%	0.05%	0.0%	0.01%	0.03%
Competitor 3	100.0%	100.0%	99.9%	0.22%	0.18%	0.03%	0.05%	0.02%
Alpha MEM Earle's Salts + 10% FBS	100.0%	100.0%	100.0%	0.63%	2.49%	0.02%	0.04%	0.22%

Percent of population positive for surface marker expression are grouped by expected positive markers (CD73, CD90, CD105) and negative markers (CD34, CD45, CD14, CD19, HLA-DR) for MSCs. The value represents the average of the triplicate measurements from a single donor.



MSCs grown for 2 passages in PRIME-XV MSC Basal XSFM supplemented with 5% hPL were assessed for trilineage differentiation potential. The MSCs successfully differentiated into chondrogenic, adipogenic, and osteogenic lineages upon culture in the respective differentiation media (**Figure 5**).

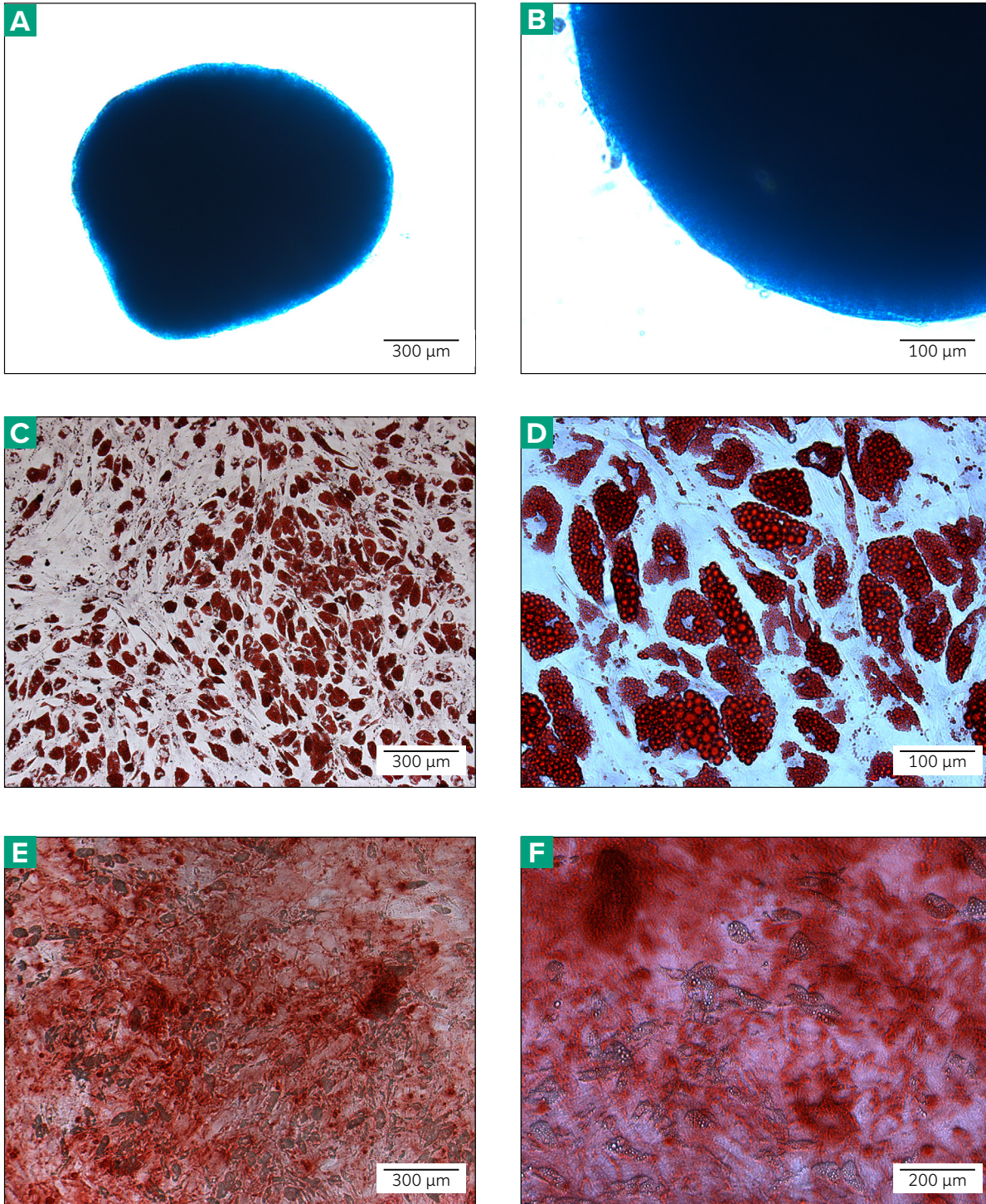


Figure 5. Trilineage differentiation potential maintained by MSCs cultured in PRIME-XV MSC Basal XSFM supplemented with 5% hPL. The MSCs cultured in PRIME-XV MSC Basal XSFM successfully differentiated into (**A, B**) chondrocytes, (**C, D**) adipocytes, and (**E, F**) osteocytes when cultured in the respective commercial differentiation media.

PRIME-XV MSC Basal XSFM was tested with research grade hPL (PLTMax). Compared to PRIME-XV MSC Expansion XSFM, 5% supplementation of PLTMax into PRIME-XV MSC Basal XSFM resulted in a similar level of MSC expansion over 4 passages (**Figure 6**).

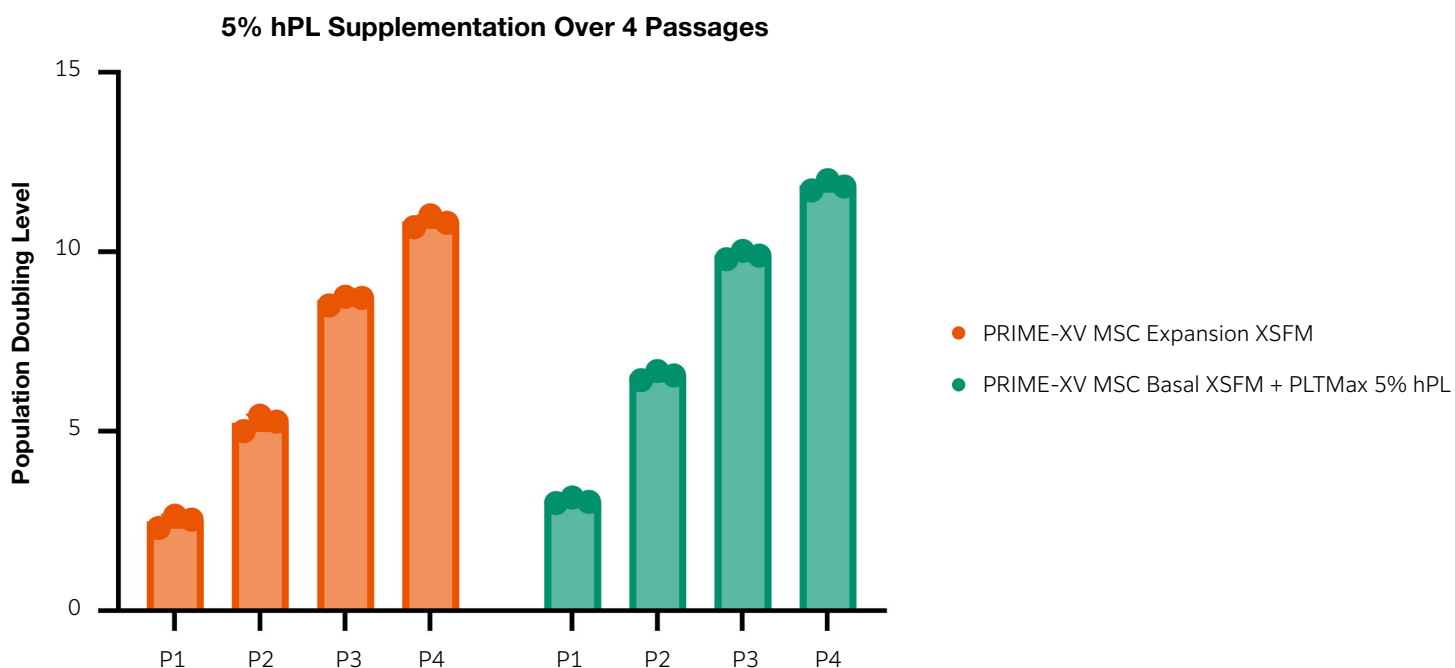


Figure 6. PRIME-XV MSC Basal XSFM supplemented with PLTMax 5% hPL supports robust expansion of hMSCs.

PRIME-XV MSC Basal XSFM supplemented with 5% hPL (PLTMax Research Grade, Mill Creek) achieves similar MSC growth compared to PRIME-XV MSC Expansion XSFM.

CONCLUSION

In this study we demonstrated that PRIME-XV MSC Basal XSFM enables robust expansion when supplemented with Mill Creek's RUO and GMP hPLs. While Alpha MEM remains an established option for expanding MSCs to therapeutic levels, PRIME-XV MSC Basal XSFM supports at least a 4-fold greater cell expansion when supplemented with 5% hPL.

This medium offers therapy developers a cost-effective approach for maximizing MSC yield through hPL supplementation. PRIME-XV MSC Basal XSFM supplemented with hPL maintains characteristic surface marker expression and trilineage differentiation potential that supports the generation of high-quality MSCs suitable for clinical applications.



fujifilmbiosciences.fujifilm.com

FUJIFILM Biosciences – Corporate

2501 Pullman St., Santa Ana, Suite 201,
CA 92705 USA

Phone: 1 (949) 261-7800

Toll Free: 1 (800) 437-5706

Fax: 1 (949) 261-6522

Support: getinfo@fujifilm.com