A Versatile and Reliable Basal Medium for MSC Expansion: PRIME-XV MSC Basal XSFM with Human Platelet Lysate

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INTRODUCTION

Mesenchymal stromal/stem cells (MSCs) have emerged as promising candidates for a variety of clinical applications, particularly in regenerative medicine and autoimmune diseases. Due to its low immunogenic potential, allogeneic approaches can be utilized for MSC-based therapies, enabling scale-up manufacturing that can produce sufficient number of cells to treat multiple patients from a single donor. However, manufacturing challenges still exist in growing a clinically-relevant number of cells while maintaining cell potency. One of the keys in achieving a robust and reproducible manufacturing process is using the right cell culture media.

Human platelet lysate (hPL) has become increasingly popular as a replacement for fetal bovine serum (FBS) as a supplement for enhancing MSC expansion. Owing to its manufacturing process of pooling human platelets from multiple donors, hPL achieves consistent lot-to-lot performance in supporting MSC growth. While classical media such as Alpha MEM can be paired with hPL, an optimized MSC basal medium designed to be used with hPL can offer a significant improvement in MSC expansion while maintaining cell quality. To address this need, FUJIFILM Biosciences recently launched PRIME-XV MSC Basal XSFM. This MSC-specific serum- and xeno-free basal medium maximizes the outcome of hPL supplementation in supporting human MSC expansion and further enables manufacturing at scale necessary for clinical applications in a cost-effective manner. Here, we demonstrate the performance of the medium in maximizing MSC yield while maintaining cell quality.

MATERIALS AND METHODS

Cell Source: Human MSCs from bone marrow, adipose tissue, and umbilical cord were purchased from commercially-available sources.

Cell Culture Reagents:

- PRIME-XV MSC Basal XSFM (FUJIFILM Biosciences, Catalog # 91235G)
- PRIME-XV MSC Expansion XSFM (FUJIFILM Biosciences, Catalog # 91149)
- Alpha MEM with Nucleosides (FUJIFILM Biosciences, Catalog # 9144)
- Alpha MEM without Nucleosides (FUJIFILM Biosciences, Catalog # 9142)
- Commercially-available MSC basal and complete media
- PLTGold-GI Clinical Grade Human Platelet Lysate (Mill Creek, Catalog # PLTGold27GMP-GI)
- PLTMax Research Grade Human Platelet Lysate (Mill Creek, Catalog # PLTMax27R)
- Commercially-available fetal bovine serum (FBS)
- PRIME-XV Human Fibronectin (FUJIFILM Biosciences, Catalog # 31002)

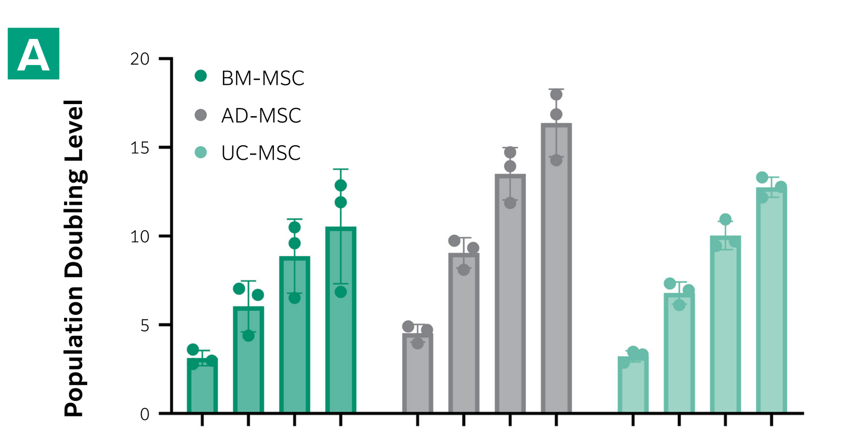
Cell Culture Conditions:

- MSCs were cultured with hPL (PLTGold-GI or PLTMax) or FBS grown on tissue culture (TC)-treated plates without coating
- MSCs cultured with fibronectin coating, where instructed by media manufacturer
- Media were replaced every 2–3 days
- Cells passaged at 70–85% confluence
- Trilineage differentiation was performed using commercial kits per manufacturer's instructions

Analytical Methods:

- Cell counting performed using Cellaca MX cell counter
- Surface marker analysis performed by antibody staining and flow cytometry
- Differentiated cells stained with: Alcian Blue (chondrogenesis), Alizarin Red S (osteogenesis), Oil Red O (adipogenesis) and assessed by phase contrast microscopy

RESULTS



B

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%

Figure 1. PRIME-XV MSC Basal XSFM supplemented with 5% hPL successfully supported expansion of various sources of hMSCs. PRIME-XV MSC Basal XSFM was supplemented with 5% hPL (PLTGold-GI) to assess expansion of human MSCs from 3 different tissue origins: bone marrow (BM), adipose tissue (AD), and umbilical cord (UC). **(A)** Across 4 passages, PRIME-XV MSC Basal XSFM supplemented with hPL supported robust expansion of all tested MSCs. Each data point represents the average of a single donor, with a total of 3 donors tested per tissue origin. **(B)** The expanded MSCs displayed characteristic MSC surface markers as assessed by flow cytometry, demonstrating that the medium supports the maintenance of MSC phenotype.

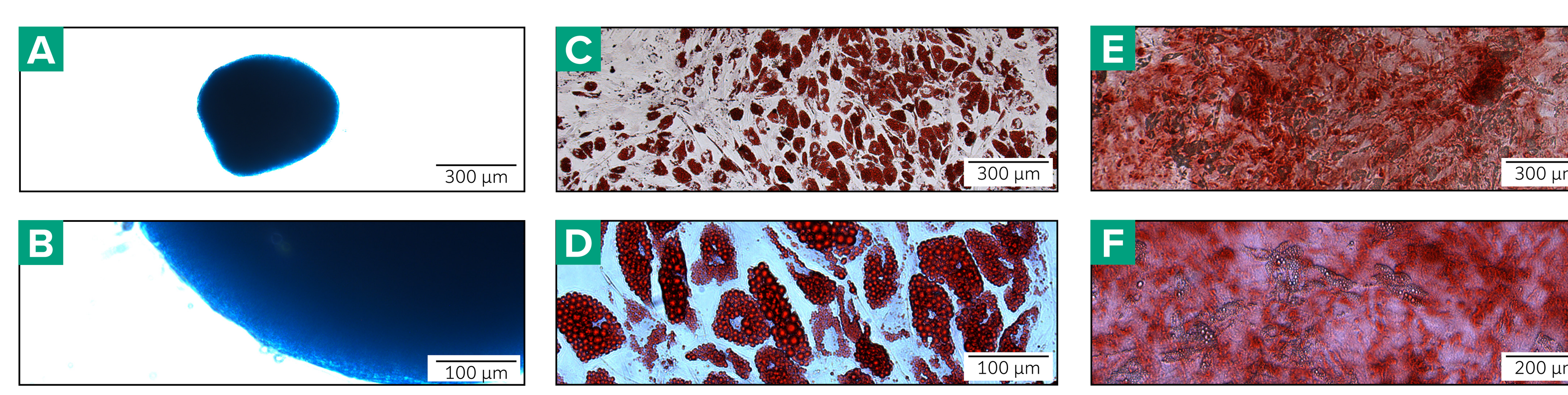


Figure 5. Trilineage differentiation potential is maintained by MSCs cultured in PRIME-XV MSC Basal XSFM supplemented with 5% hPL. MSCs grown for 2 passages in PRIME-XV MSC Basal XSFM supplemented with 5% hPL were assessed for trilineage differentiation potential. The MSCs cultured in PRIME-XV MSC Basal XSFM successfully differentiated into **(A, B)** chondrocytes, **(C, D)** adipocytes, and **(E, F)** osteocytes when cultured in the respective commercial differentiation media.

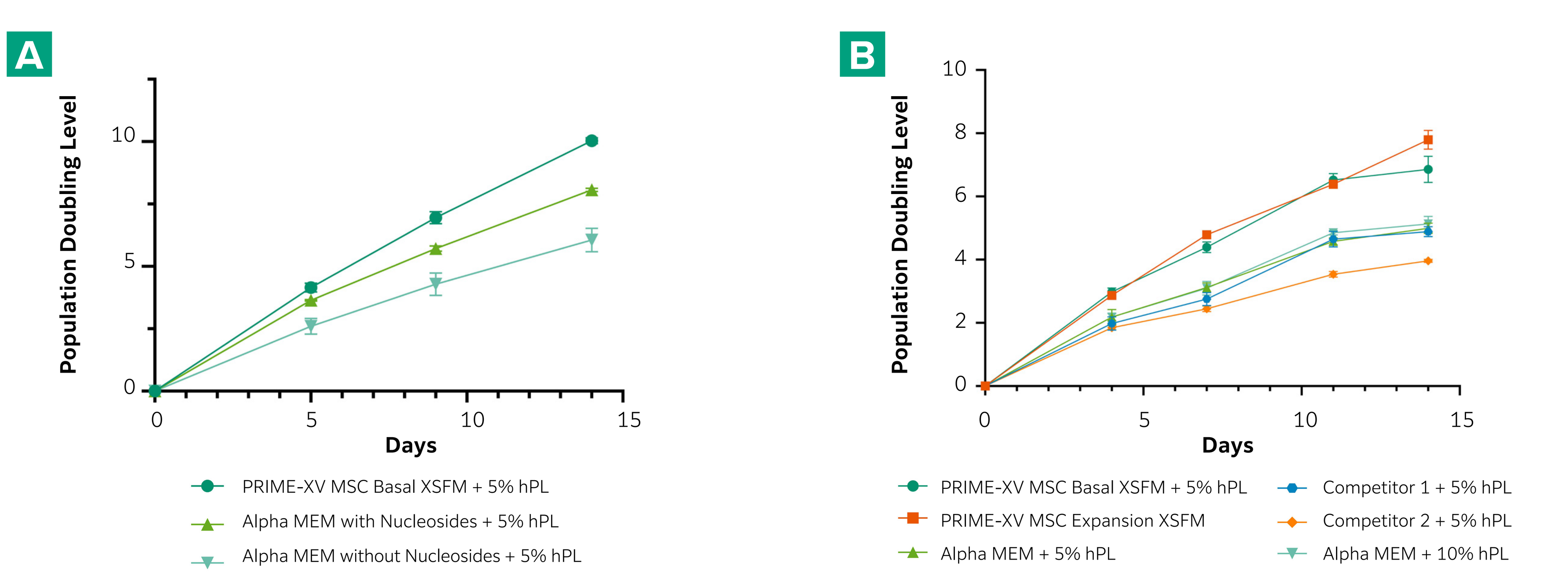


Figure 2. PRIME-XV MSC Basal XSFM supplemented with 5% hPL outperformed classical and commercial media supplemented with 5% hPL. (A) After 14 days of MSC expansion, MSC Basal XSFM supplemented with 5% hPL (PLTGold-GI) outperformed Alpha MEM with Nucleosides supplemented with 5% hPL by ~2 total population doublings (i.e., ~4-fold greater expansion) and Alpha MEM without Nucleosides supplemented with 5% hPL by ~4 total population doublings (i.e., ~16-fold greater expansion). **(B)** MSC Basal XSFM supplemented with 5% hPL outperformed commercially-available MSC basal media supplemented with 5% hPL.

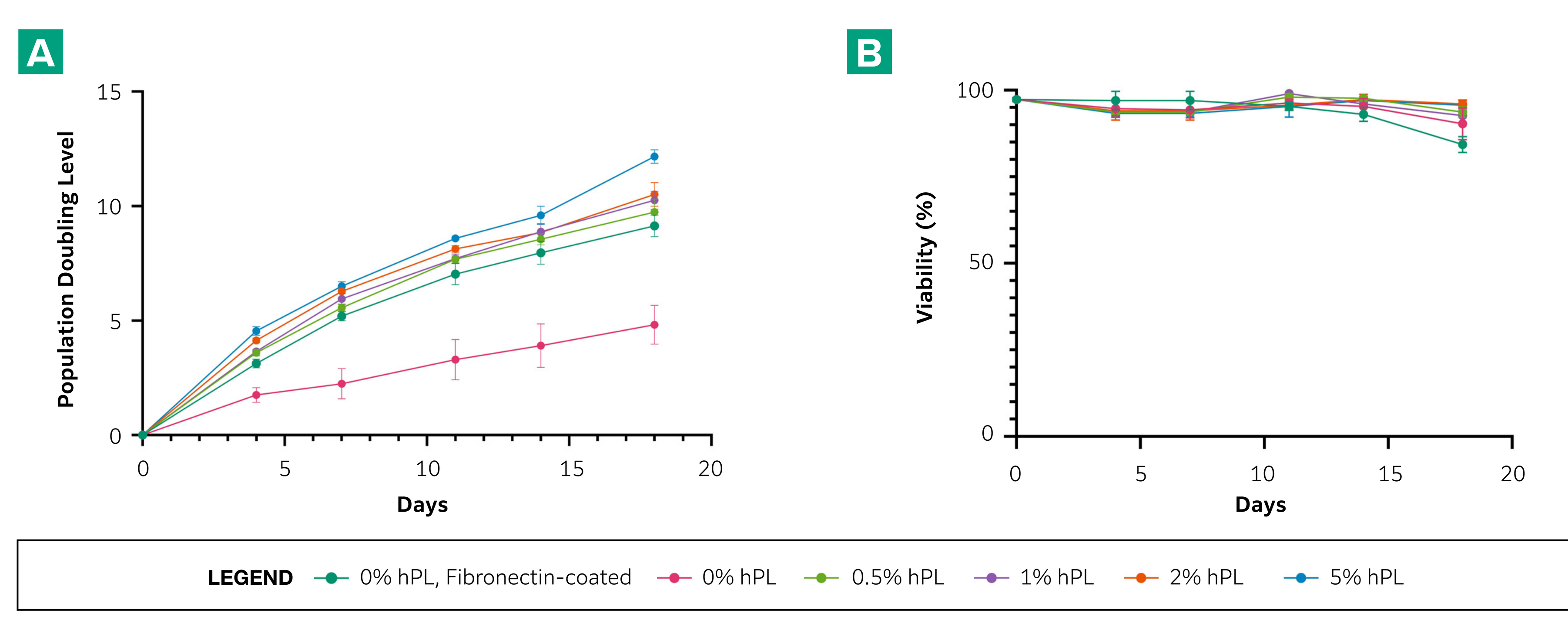


Figure 6. Supplementation of hPL to PRIME-XV MSC Expansion XSFM led to increased expansion while removing the need to use coating substrate. MSCs were cultured using PRIME-XV MSC Expansion XSFM without hPL (with and without fibronectin-coated wells) or containing 0.5%–5% hPL (all without fibronectin-coated wells). The supplementation of hPL resulted in **(A)** increased proliferation in a dose-dependent manner up to the highest concentration tested and **(B)** maintained high viability across 5 passages.

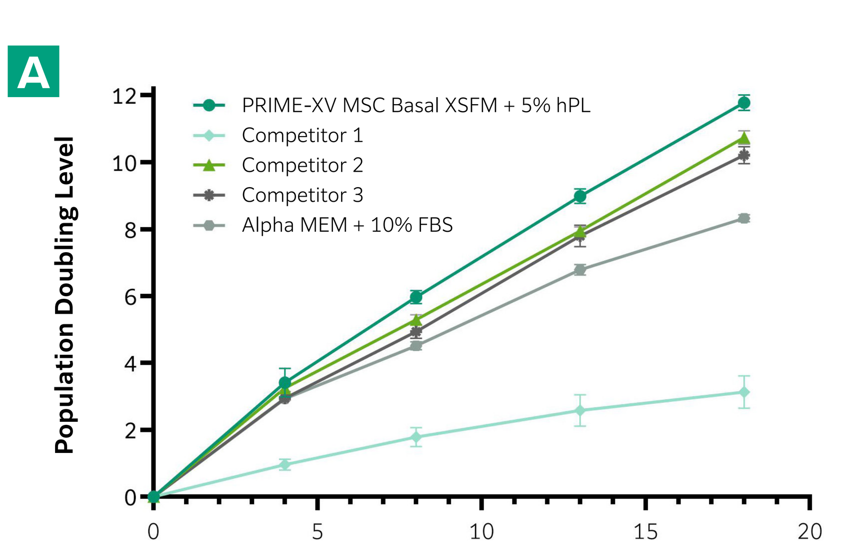


Figure 3. PRIME-XV MSC Basal XSFM supplemented with 5% hPL outperformed competitor MSC media. Over the course of 4 passages, PRIME-XV MSC Basal XSFM with 5% hPL (PLTGold-GI) outperformed commercially available complete MSC media as well as Alpha MEM supplemented with 10% FBS.

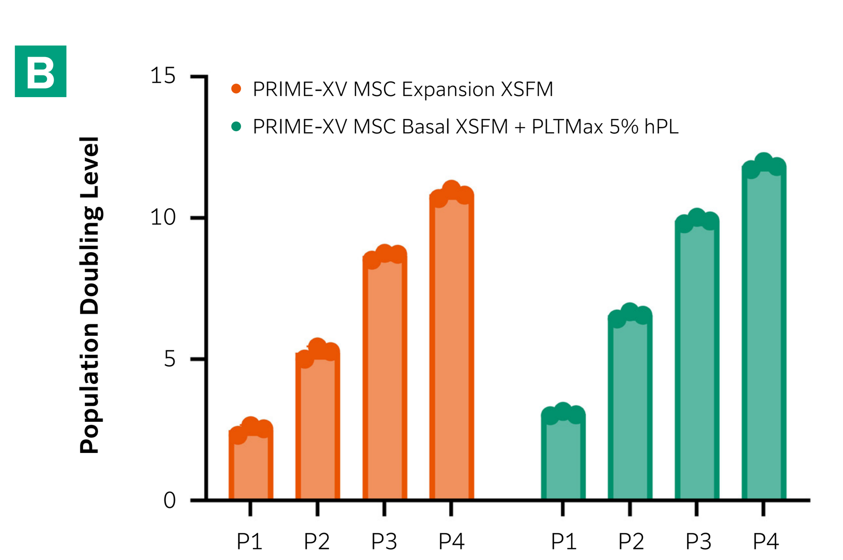


Figure 4. PRIME-XV MSC Basal XSFM supplemented with 5% PLTMax supports robust expansion of hMSCs. Compared to PRIME-XV MSC Expansion XSFM, 5% supplementation of PLTMax Research Grade hPL into PRIME-XV MSC Basal XSFM resulted in a similar level of MSC expansion over 4 passages.

SUMMARY

The use of hPL for clinical expansion of MSCs has become increasingly popular due to high performance, lot-to-lot consistency, and regulatory acceptance. In this experiment, we demonstrate how our latest MSC medium, PRIME-XV MSC Basal XSFM, can be paired with hPL to address current challenges in scaling MSC manufacturing processes in a consistent and cost-efficient manner.

Key findings include:

- Achieving robust MSC expansion using PRIME-XV MSC Basal XSFM supplemented with 5% hPL
- Maintaining high viability and potency of expanded MSCs

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