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Human Platelet Lysate (hPL) Improves Expansion of MSCs

Using PRIME-XV MSC Expansion XSFM

APPLICATION NOTE

INTRODUCTION

Mesenchymal stromal/stem cells (MSCs) exhibit exceptional immunomodulatory properties, making them suitable for cell-based therapeutics development. Traditionally, MSCs have been cultured in media supplemented with serum, usually derived from bovine or human sources. Human platelet lysate (hPL) has been shown to promote cell proliferation, migration, and differentiation, leading to improved cell culture outcomes when compared to bovine serum. hPL contains a higher concentration of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and insulin-like growth factor (IGF)—which play crucial roles in cell growth and proliferation. Additionally, hPL can promote cell adhesion, thus eliminating the need to coat the culture surface with an adhesion protein such as fibronectin.

In this study, we investigated the effect of supplementing PRIME-XV MSC Expansion XSFM with 0.5% to 5% hPL on the expansion of MSCs. The study was performed using standard tissue culture (TC) plates without any adhesion proteins (except in the control which used fibronectin). The effect of hPL addition on cell growth and viability, as well as marker expression, was evaluated. The results presented here indicate that the addition of hPL to PRIME-XV MSC Expansion XSFM promotes MSC adhesion and expansion.

PRIME-XV MSC Expansion XSFM is a xeno-free and serum-free complete medium optimized for the maintenance and expansion of purified human MSCs. PRIME-XV MSC Expansion XSFM supports the expansion of MSCs derived from human adipose, human bone marrow, and human umbilical cord, and maintains cell quality even after extended culture. PRIME-XV MSC Expansion XSFM is available in a ready-to-use format, but can be supplemented with cytokines, growth factors, or other components, if needed for the specific application.

MATERIALS

Reagents and Equipment		
• **PRIME-XV MSC Expansion XSFM** (FUJIFILM Biosciences, Catalog # 91149), • **PRIME-XV Human Fibronectin** (FUJIFILM Biosciences, Catalog # 31002) • **Bone Marrow–Derived Mesenchymal Stem Cells (BM-MSC)** (American Type Culture Collection, Catalog # PCS-500-010) • **PLTGold-GI Clinical Grade Human Platelet Lysate** (Mill Creek Life Sciences, Catalog # PLTGold27GMP-GI)	• **Invitrogen LIVE/DEAD Fixable Blue Dead Cell Stain** (Thermo Fisher Scientific, Catalog # L34962) • **ViaStain AOPI Staining Solution** (Revvity, Catalog # CS2-0106-25ML) • **Cellaca MX High-throughput Cell Counter** (Revvity) • **BD FACSymphony A5 Cell Analyzer** (BD Bioscience)	• **CD105** (BioLegend, Catalog # 323206) • **CD90** (BioLegend, Catalog # 328114) • **CD73** (BioLegend, Catalog # 344010) • **CD34** (BioLegend, Catalog # 343604) • **HLA-DR** (BioLegend, Catalog # 307645) • **CD14** (BioLegend, Catalog # 612763) • **CD45** (BioLegend, Catalog # 304028)

METHODS

Media preparation

PRIME-XV MSC Expansion XSFM was aliquoted and supplemented with hPL at concentrations of 0.5%, 1%, 2%, and 5% to prepare the test samples. A control sample without hPL was also prepared.

Cell expansion and passaging

MSCs were seeded in TC-treated plates using the control and hPL-supplemented PRIME-XV MSC Expansion XSFM samples. Wells for control with fibronectin were precoated with 2 µg/cm^2 fibronectin. Each condition was tested in triplicate wells. MSCs were cultured at 37°C in a humidified incubator with 5% CO_2. The cells were maintained for 5 passages, where the cells were fed with fresh media every 2 to 3 days and expanded for up to 4 days before passaging.

Cell count and viability

The expanded MSCs were washed with Dulbecco's Phosphate-Buffered Saline (DPBS) without $Ca2^+$ and $Mg2^+$, then dissociated using TrypLE. The dissociated MSCs were quenched with the corresponding XSFM sample and centrifuged at 300 x g for 5 minutes. The cells were resuspended in the corresponding XSFM sample and aliquoted for cell counting and viability measurements. Cell suspension was mixed with ViaStain AOPI Staining Solution and counted using the Cellaca MX High-throughput Cell Counter.

Marker expression

To prepare for flow cytometry to measure marker expression, the cells were washed in flow buffer and stained with a 1:100 dilution of LIVE/DEAD Fixable Blue Dead Cell Stain. The cells were incubated for 30 minutes at 4°C and washed with flow buffer before staining with a 1:500 dilution of antibodies for CD105, CD90, CD73, CD34, HLA-DR, CD14, and CD45. The cells were incubated for 30 minutes at 4°C, washed with flow buffer 3 times, and then resuspended in flow buffer. The samples were measured using the BD FACSymphony A5 to quantify the expressed markers.

RESULTS

The 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 total population doubling levels (PDL) and the percent viability of cells over the course of 5 passages are shown in **Figures 1A and 1B**, respectively.

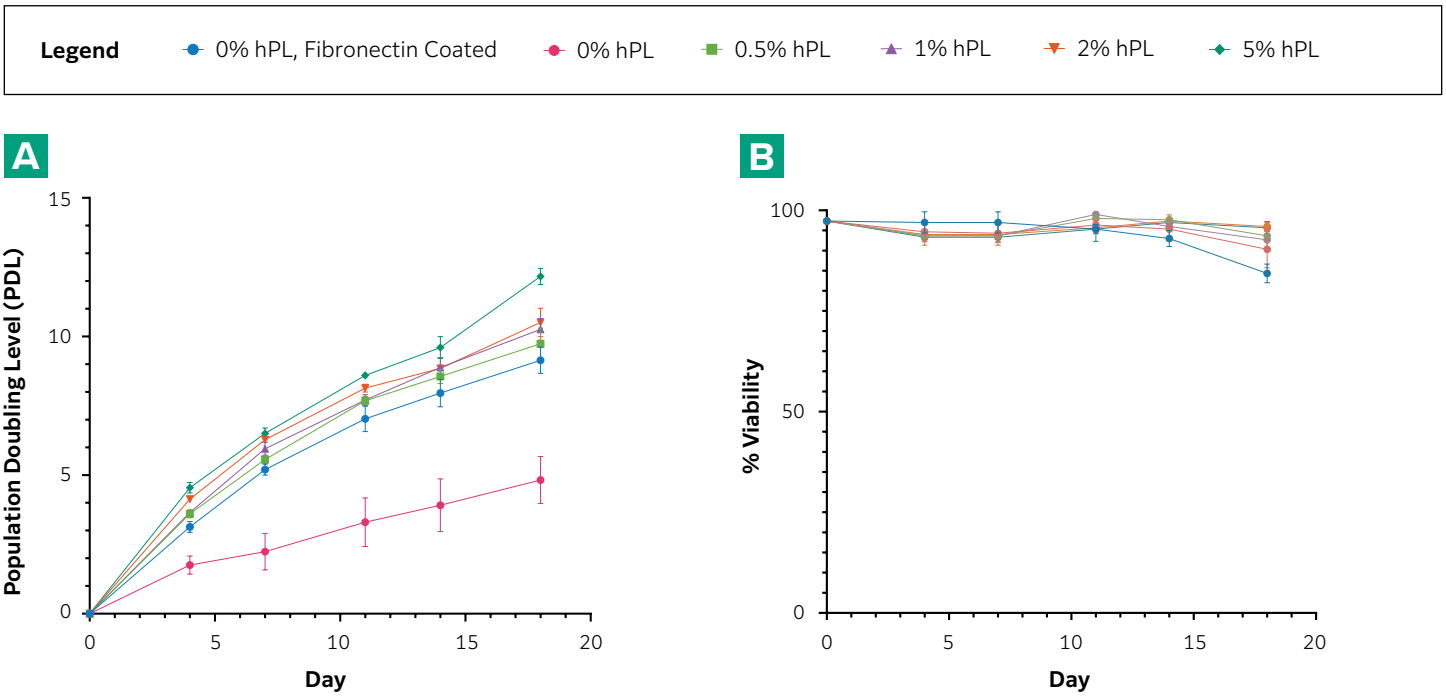

Figure 1A. The total population doublings over 5 passages. Viable cell density counts were used to calculate total population doublings. Population doubling level = $\log_2(Cf/Ci)$, where Cf is the total cell count at collection and Ci is the total cell count at seeding.

Figure 1B. The viability of MSCs over 5 passages. Cells were analyzed at each passage for viability.

Surface marker expression levels were assayed using antibody labeling and flow cytometry. Quantification of the MSCs in the population was performed by gating specific cell populations according to their CD73, CD90, CD105, CD14, CD34, CD45, and HLA-DR expression. The results for the marker expression analysis are shown in **Table 1**.

Table 1. Flow cytometry analysis for marker expression.

	Positive Markers			Negative Markers			
Sample	CD73	CD90	CD105	CD14	CD34	CD45	HLA-DR
Day 0 Control	**99.9%**	**99.9%**	**99.8%**	0.0%	0.0%	0.0%	0.0%
0% hPL, Fibronectin Coating	**99.8%**	**99.8%**	**98.4%**	0.1%	0.4%	0.2%	0.4%
0% hPL	**99.5%**	**99.4%**	**97.8%**	0.1%	0.4%	0.2%	0.5%
0.5% hPL	**99.9%**	**99.6%**	**96.4%**	0.4%	0.0%	0.0%	0.0%
1% hPL	**100 .0%**	**99.6%**	**97.1%**	0.8%	0.0%	0.0%	0.0%
2% hPL	**100 .0%**	**99.7%**	**96.4%**	1.2%	0.0%	0.0%	0.0%
5% hPL	**100 .0%**	**99.1%**	**97.0%**	1.7%	0.0%	0.0%	0.0%

The cells were analyzed using a panel of positive and negative markers after 5 passages. Day 0 Control refers to cells labeled and analyzed prior to initial seeding.

CONCLUSION

This study examined the effect of supplementation of PRIME-XV MSC Expansion XSFM with hPL (0.5% – 5%) on its ability to support MSC adhesion and expansion. The results show that the addition of hPL to PRIME-XV MSC Expansion XSFM resulted in significantly higher PDLs compared to the no-hPL condition with fibronectin-coated wells in a dose-dependent manner. Conversely, a significantly lower PDL was achieved for the no-hPL condition without fibronectin-coated wells. The combined data indicate that the addition of hPL (even at the lowest concentration tested) can eliminate the need to precoat the culture surface to support MSC adhesion and that the addition of hPL improves MSC adhesion and/or proliferation in a dose-dependent manner up to the highest concentration tested. All conditions with hPL showed comparable viability and consistent cell growth across 5 passages, regardless of the hPL amount. The cells expanded with PRIME-XV MSC Expansion XSFM containing hPL demonstrated similar surface markers to those grown in PRIME-XV MSC Expansion XSFM without hPL. Overall, the results demonstrate that supplementing PRIME-XV MSC Expansion XSFM with hPL improves MSC adhesion and expansion without affecting cell phenotype.

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