Human Platelet Lysate Produced at Industrial Scale for use as Fetal Bovine Serum Replacement in MSC Cell Therapies

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Introduction

Limitation of FBS: The use of fetal bovine serum (FBS) for clinical manufacturing of stem cell products poses risks including the potential for viral and prion transmission and the possibility of adverse immunological reactions.

Limitation of Traditional Method: Traditionally, HPL has been prepared by individual laboratories in small batches using protocols that differ in the number of platelet units pooled, the processing of platelets, and the requirement for heparin. These differences can significantly impact stem cell growth, morphology, and functionality.

PLUS™ Human Platelet Lysate (hPL): To address these issues, we have developed a highly standardized, industrial-scale production process for our PLUS™ hPL (up to 100 L lot size) that uses good manufacturing practices (GMP) to produce a viable, xenogeneic-free alternative to FBS.

Objective: Our primary focus for this study was to assess the capacity of PLUS™ hPL to replace FBS for manufacturing of several cellular products, including human bone marrow-derived stromal cells (hMSCs) and human adipose-derived stromal cells (hASCs), human neonatal dermal fibroblasts (NHDF), etc.

PLUS™ Human Platelet Lysate	Clinical	Research	
	GMP-PLUS™	PLUS™	MSC-Qualified PLUS™
Manufacturing process	Fully closed	Fully closed	Fully closed
Filling process	Fully closed	Controlled environment	Controlled environment
Distribution	Cryobags	Bottles	Bottles
Released SOPs for all processes	Yes	Partial	Partial
Performance testing	Confirmed by hMSC outgrowth	None	Confirmed by hMSC outgrowth

Methods

Platelet Lysate Manufacturing: Expired platelet units were acquired from FDA-registered AABB accredited blood banks and lysed using a freeze-thaw process. Cell debris and clotting factors were removed. Each lot was produced by pooling >100 donors.

ELISA: Growth factor concentrations were quantified using human-specific ELISA kits (R&D Systems).

Tissue Origin: 9 different donors of fresh human bone marrow (hBM) from the posterior iliac crest were obtained from Lonza or AllCells. 6 different donors of fresh human lipoaspirate tissue from the abdomen or thigh were obtained from ZenBio.

CFU Assay: Mononuclear cells (MNCs) were isolated via Ficoll gradient centrifugation and plated into standard tissue culture flasks at a density of 50,000 MNCs/cm^2. After culturing for 7 days, colonies were fixed with methanol and stained with Giemsa solution for visual quantification.

Cell Culture: Isolated hMSCs and hASCs were seeded at 1,000 cells/cm^2 in αMEM supplemented with MSC-Qualified FBS (Invitrogen) or different concentrations of PLUS™ hPL with medium change every third day, and harvested with trypsin when ~85% confluency was reached.

Flow Cytometry: Viable cell counting and immunophenotyping were performed using a C6 Flow Cytometer (BD Accuri). An MSC Phenotyping Kit (Miltenyi) was used for MSC surface marker identification per ISCT guidelines.

Immunosuppression: Carboxyfluorescein succinimidyl ester (CFSE, Invitrogen)-labeled human peripheral blood mononuclear cells (PBMCs) were cultured at 400,000 cells/well in a 24-well plate in 10% FBS RPMI. T lymphocytes were stimulated to proliferate using human CD3/CD28 Dynabeads (Invitrogen) in the absence or presence of MSCs. T cell proliferation was determined 4 days later by flow cytometry analysis of CFSE fluorescence intensity.

Contact

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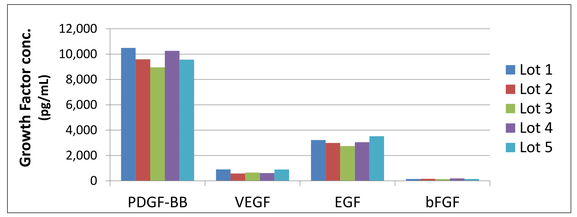
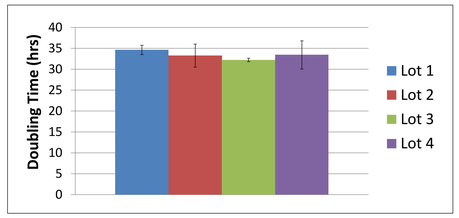
Results

Lot-to-lot consistency of PLUS™ hPL produced at industrial scale

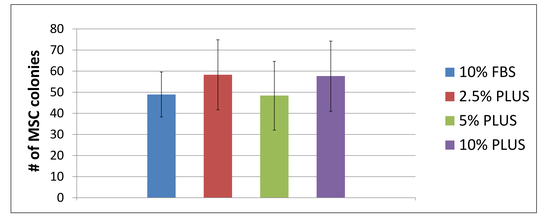


Data represents the individual concentrations of four important growth factors (PDGF-BB, VEGF, EGF and FGF basic) in five different lots of PLUS™ hPL, as quantified by ELISA. Growth factor levels are comparable between each individual lot.



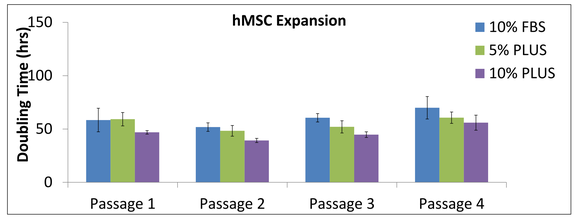
Data represents the average doubling time of P3 hMSCs from three donors cultured in αMEM supplemented with four different lots of PLUS™ human platelet lysate (all at 5% v/v). Doubling time is NOT lot dependent ($p > 0.05$).

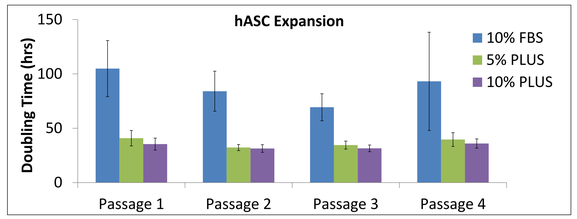
Efficient isolation of hMSCs with PLUS™ hPL



Data represents the average number of plastic-adherent hMSC colonies obtained from 9 different donors of hBM. The number of colonies was comparable among different concentrations of PLUS™ hPL and the FBS control.

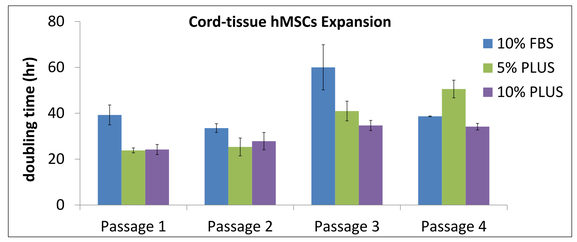
Ex *vivo* expansion of hMSCs and hASCs with PLUS™ hPL





Data represents the average doubling time for hMSCs (top, n= 9 donors) and hASCs (bottom, n = 6 donors) over multiple passages in αMEM supplemented with different concentrations of FBS or PLUS™ hPL.

Cord-tissue derived MSCs also expand with PLUS™ hPL

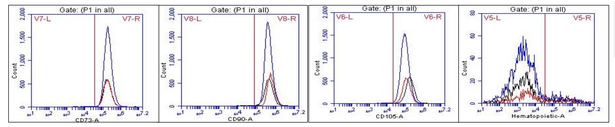


In a preliminary experiment with n=1, cord-tissue derived hMSCs were able to expand at a slightly faster speed in PLUS™ hPL supplemented medium as compared to FBS over multiple passages.

MSC phenotype maintenance with PLUS™ hPL

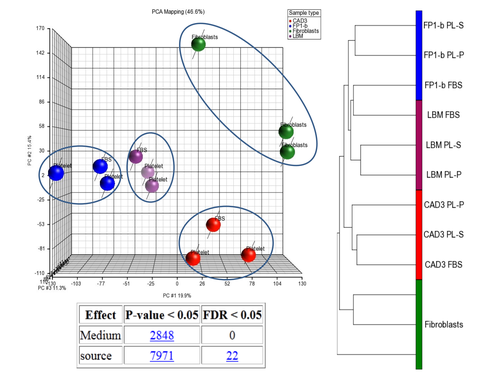
	Average from 9 donors of hMSCs			
	% CD90+	% CD105+	% Hematopoietic+	% CD73+
10% FBS	100±0.03	100±0.03	2.07±0.34	99.8±0.05
5% PLUS	98.1±3.29	98.1±3.28	1.60±0.25	98.0±3.22
10% PLUS	99.1±1.46	99.1±1.49	1.13±0.19	99.0±1.49

	Average from 6 donors of hASCs			
	% CD90+	% CD105+	% Hematopoietic+	% CD73+
10% FBS	99.6±0.21	99.6±0.34	0.50±0.12	99.9±0.06
5% PLUS	99.8±0.12	98.5±0.65	0.35±0.06	100±0.06
10% PLUS	99.9±0.09	98.3±0.51	0.28±0.05	100±0.04



After 4 passages of culture, both FBS and PLUS™ hPL expanded hMSCs and hASCs maintained their phenotypes.

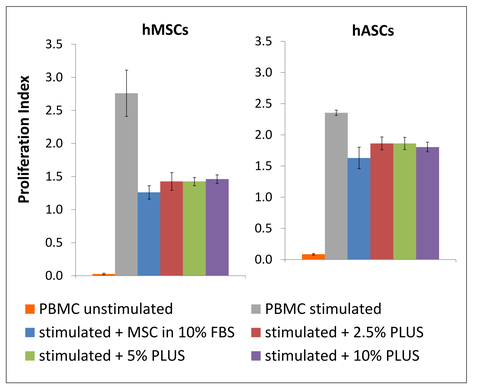
hMSCs cultured in PLUS™ or FBS have similar global gene expression profiles



Effect	P-value < 0.05	FDR < 0.05
Medium	2848	0
source	7971	22

Transcriptome analysis of BMSCs prepared from 3 different sources of starting marrow cells: healthy subject marrow cells isolated by density gradient separation (blue), unfractioned marrow aspirate from a healthy subject (purple) and unfractioned marrow aspirate from a cadaver (red). Each of the 3 types of marrow cells were culture in three different media: media supplement with FBS, media supplemented with platelet lysate-P and media supplemented in platelet lysate-S. Transcriptome analysis of fibroblasts are shown as a control (green).

Immunosuppressive activity with PLUS™ hPL



Following a 4-day exposure of PBMCs to CD3/CD28 activation, T cells proliferated robustly in the absence of hMSCs, whereas both PLUS™ hPL and FBS-expanded hMSCs or hASCs at a 1:10 dilution could significantly reduce the proliferation at the same level ($p<0.05$). The proliferation index was calculated as log(FInd/MFIall)/log(2), where MFIall = median fluorescence intensity of all viable T cells and FInd = peak fluorescence intensity of the viable non-divided cells. Left: hMSCs, right: hASCs; n=6 donors for both.

Discussion

- Human platelet lysate (hPL) can be produced at an industrial scale (platelet units from >100 donors pooled) resulting in a safe product with minimal lot-to-lot variation.
- hPL can be used throughout the MSC manufacturing process from isolation of cells (via plastic adherence) to *ex vivo* expansion to cryopreservation.
- Both hMSCs and hASCs expand extensively in hPL with significantly higher yields than FBS. The difference is much greater for hASCs than hMSCs.
- hMSCs and hASCs expanded in hPL over multiple passages maintain their spindle morphology and expression of characteristic surface markers.
- The immunosuppressive activity is comparable between hPL and FBS-expanded hMSCs/hASCs due to hPL's minimal fibrinogen concentration.