Clinical-Grade Human Platelet Lysate Produced at Industrial Scale for Isolation, Expansion, and Cryopreservation of Mesenchymal Stromal Cells

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Introduction

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. Human platelet lysate (HPL) has emerged as a viable, xenogeneic-free alternative. 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 multipotency. To address this issue, we have developed a highly standardized, industrial-scale production process for our PLUS™ HPL using good manufacturing practices (GMP). Our focus for this study was to assess the capacity of PLUS™ HPL to serve as a media supplement for the isolation, *ex vivo* expansion, and cryopreservation of human bone marrow (hBM) mesenchymal stromal cells (MSCs).

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 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 factors were quantified via enzyme linked linked immunosorbent assay (ELISA) using Quantikine ELISA kits from R&D Systems.

Bone marrow: Fresh human bone marrow (hBM) from the posterior iliac crest was obtained from either Lonza (Walkersvile, MD) or AllCells (Emeryville, CA).

CFU assay: Mononuclear cells (MNCs) were isolated from hBM via gradient centrifugation using Ficoll Paque (GE Healthcare) and plated at a density of $1.25x10^2$ MNCs/cm^2 into standard T25 culture flasks or Corning CellBIND® flasks (serum-free condition only). After culturing for 9 days, colonies were either fixed with methanol and stained with Giemsa solution for visualization/quantification or sub-cultured.

Cell Culture: Cells were cultured in αMEM media supplemented with MSC-Qualified FBS (Invitrogen) or PLUS™ or in Corning stemgro® serum-free medium. For each passage (beginning with P0 plastic-adherent colonies), cells were seeded at a density of 1,000 cells/cm^2 into T25 flasks and harvested at day 6 using trypsin-EDTA. Media changes were performed every third day.

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 identification per ISCT guidelines. Counting was carried out using 7-AAD or propidium iodide and Accucount Fluorescent Particles (Spherotech).

Contact

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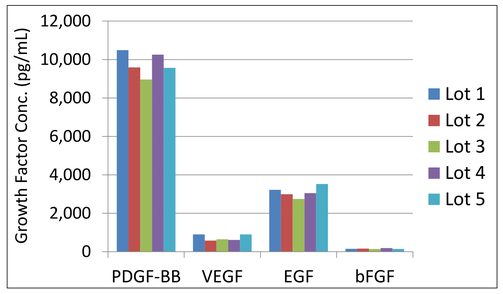
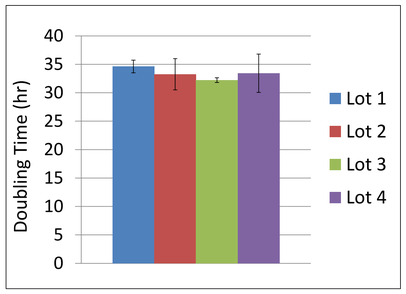
COMPASS BIOMEDICAL
AN ARTERIOCYTE COMPANY

Results

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

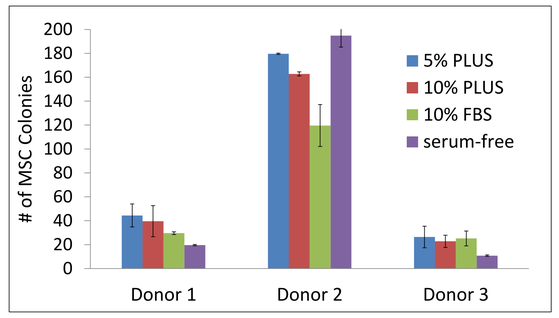


Data represents the individual concentrations of four important growth factors (PDGF-BB, VEGF, EGF and FGF basic) in five different lots of PLUS™ human platelet lysate, as quantified by ELISA. Growth factor levels are comparable for each lot.



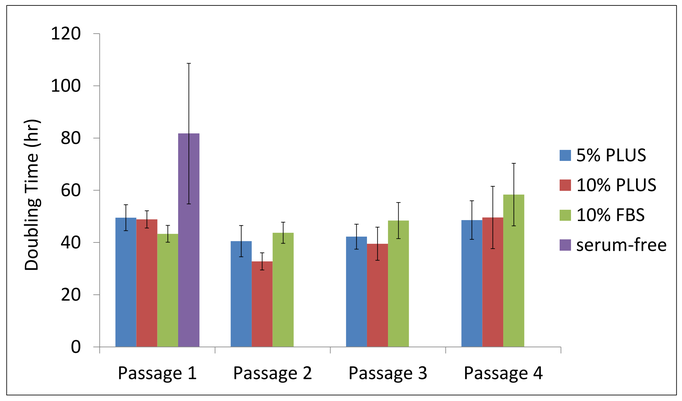
Data represents the average doubling time of P3 hBM MSCs from three donors cultured in αMEM supplemented with four different lots of PLUS™ human platelet lysate (all at 5% v/v). Doubling time is lot independent ($p > 0.05$).

Efficient isolation of MSCs with PLUS™ HPL

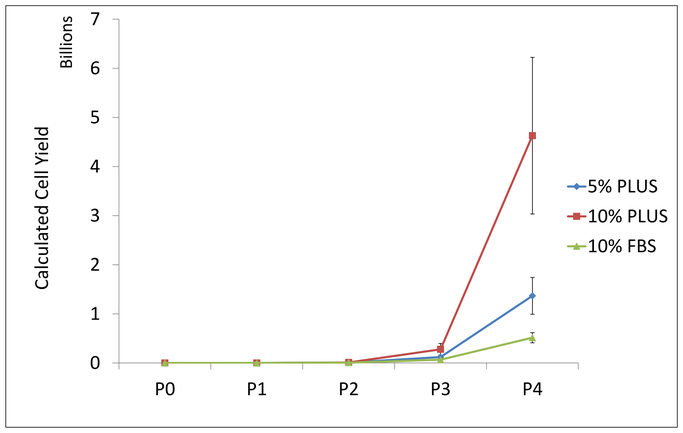


Data represents the average number of plastic-adherent MSC colonies obtained after 9 days culture of hBM MNCs in T25 flasks from three different donors. There was significant donor to donor variation, but on average culture in PLUS™ led to higher numbers of colonies.

Extensive *ex vivo* expansion of MSCs with PLUS™ HPL



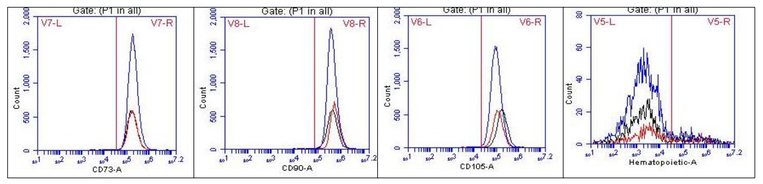
Data represents the average doubling time for hBM MSCs over multiple passages in αMEM supplemented with FBS or PLUS™ or in serum-free medium (P1 only). Data is from three different donors for P1 and six different donors for P2 through P4. Doubling time was calculated as $(t1-t2)*(\ln(2)/\ln(cell\#_{t1}/cell\#_{t2})$.



Data represents the calculated cumulative yield of hBM MSCs at each passage after culture in αMEM supplemented with FBS or PLUS™ from an initial starting dose of 50,000 MSCs. Data is from three different donors for P1 and six different donors for P2 through P4.

MSC phenotype maintenance with PLUS™ HPL

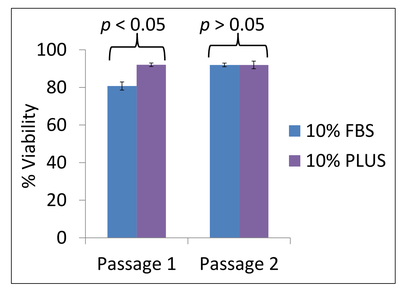
	% CD90+	% CD105+	% Hematopoietic+	% CD73+
10% FBS	100 ± 0.029	100 ± 0.029	2.07 ± 0.34	99.8 ± 0.050
5% PLUS	98.1 ± 3.29	98.1 ± 3.28	1.60 ± 0.25	98.0 ± 3.22
10% PLUS	99.1 ± 1.46	99.1 ± 1.49	1.13 ± 0.19	99.0 ± 1.49



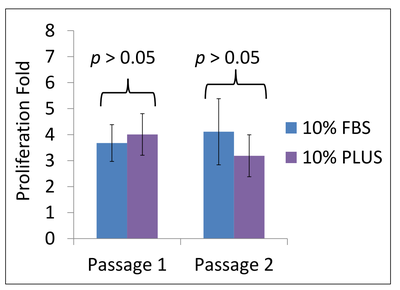
10% FBS
5% PLUS™
10% PLUS™

Data represents the average percentage of hBM MSCs from three different donors positively expressing MSC markers (CD73, CD90 and CD105) or hematopoietic markers (CD45, CD34, CD14 and CD20) after three passages in αMEM supplemented with either FBS or PLUS™.

Successful cryopreservation of MSCs with PLUS™HPL



Data represents the average viability of thawed P1 and P2 hBM MSCs from three different donors after culture in either 10% (v/v) FBS or PLUS™. After each passage, PLUS™ cultured cells were cryopreserved in 50% αMEM/42.5% PLUS™/7.5% DMSO (v/v) and FBS cultured cells were cryopreserved in a corresponding FBS containing solution.



Data represents the average effective proliferation fold of thawed P1 and P2 hBM MSCs from three different donors after culture in either 10% (v/v) FBS or PLUS™. Effective proliferation fold takes into account both the recovery of viable MSCs from thawing and the actual post-thaw proliferation fold.

Conclusions

- PLUS™ human platelet lysate (HPL) can be produced at Industrial scale (platelet units from >100 donors pooled) resulting in a safe product with minimal lot-to-lot variation
- PLUS™ HPL can be used throughout the human bone marrow (hBM) mesenchymal stromal cell (MSC) manufacturing process from isolation of cells (via plastic adherence) to *ex vivo* expansion to cryopreservation
- hBM MSCs expand extensively in PLUS™ HPL; when PLUS™ HPL is supplemented into media at 10% (v/v), significantly higher cell yields can be obtained compared to FBS at the same supplementation level