Performance of PLASTEM® as a Consistent Culture Supplement for Cell Therapy Manufacture

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INTRODUCTION

- Culture media supplements such as human platelet lysate (hPL), fetal bovine serum (FBS), and male AB serum (hSerAB) are essential raw materials for cell therapy manufacture.
- Supplements must be of high quality and compliant with regulatory standards, but they also present challenges due to safety concerns, potential complexity, individual batch validation and limited batch sizes[1].
- PLASTEM® (Grifols) is a consistent culture media supplement manufactured by fractionation of human plasma from >1000 donors, with a robust safety profile, consistency and scalable production. PLASTEM® combined with fibroblast growth factor (FGF) is an effective media supplement for human mesenchymal stem cell culture[2].

AIM

To evaluate the performace of PLASTEM® as a culture media supplement for cell therapy manufacture.

METHODS

Human mesenchymal stromal cells (hMSCs) were cultured in DMEM supplemented with either FBS 10%, PLASTEM® 10% + hPL 0.5%, or PLASTEM® 10% + FGF.

Human Peripheral Blood Pan-T Cells (T cells) were cultured with RPMI 1640 supplemented with either, FBS 10%, PLASTEM® 15%, or hSerAB 5%.

Cell density was determined by Trypan blue exclusion assay for hMSCs and T cells. Expansion factor was calculated as final/initial seeding concentration.

Multipotentiality assays. Osteogenesis (Alizarin Red S staining), chondrogenesis (dark-blue copper-containing dye Alcian Blue) and adipogenesis (Oil Red staining) were determined in hMSCs supplemented with FBS or PLASTEM® + hPL 0.5% after 21 days.

Immunomodulation assays. hMSCs supplemented with FBS, or with PLASTEM® 10% + hPL 0.5% were expanded for 21 days. Peripherial blood mononuclear cells (PBMCs) were stained with carboxyfluorescein succinimidyl ester, co-cultured with hMSCs and activated with phytohemagglutinin (PHA). The proliferation percentage was determined by flow cytometry.

Immunophenotyping. Expression of cell surface markers was determined using flow cytometry in cells supplemented with PLASTEM® 10% + hPL 0.5%. hMSCs were stained with anti-CD90, anti-CD73, anti-CD105, anti-CD45, anti-CD14, anti-CD19, anti-CD34, and anti-HLA-DR. T cells were stained with anti-CD3, anti-CD4, anti-CCR7, anti-CD45, anti-CD45RA and anti-CD8.

RESULTS

- In hMSCs, PLASTEM® 10% + FGF resulted in an efficient **cell proliferation** (71±9.5%) after 13-day compared with FBS (52.4±1.36%) or PLASTEM®+ hPL 0.5% (39.2±4.6%) (Figure 1A). T cells grew at similar rate in both media (fold expansion of cells after 12-day culture; FBS (54.2±7.5%), hSerAB 5% (49.2±18.6%), and PLASTEM® 15% (54.9±4.7%) (Figure 1B).

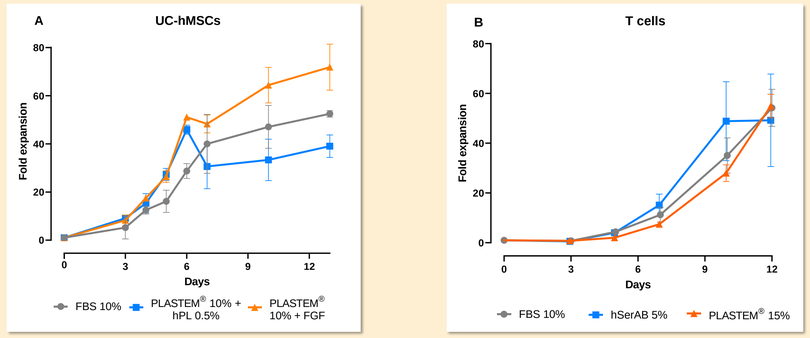

FIGURE 1. Determination of cell proliferation in (A) umbilical cord human mesenchymal stromal cells (UC-hMSCs) supplemented with FBS 10%, PLASTEM® 10% + hPL 0.5% or PLASTEM® 10% + FGF and (B) Human Peripheral Blood Pan-T Cells (T cells) supplemented with FBS, PLASTEM® 15% or hSerAB 5%. Results expressed as fold expansion between final and initial cell density (n=3).

- hMSCs cultured in PLASTEM® + hPL 0.5% supplemented media retained its phenotypic characteristics: adherence to plastic, the **multipotentiality** into osteoblasts, adipocytes or chondrocytes (Figure 2A) and the expression of **specific immune markers** CD90, CD73 and CD105 (99.9-100% expression) (Figure 2B). Negative markers for hMSCs were CD45, CD14, CD19, CD34 and HLA-DR (<5% expresssion).

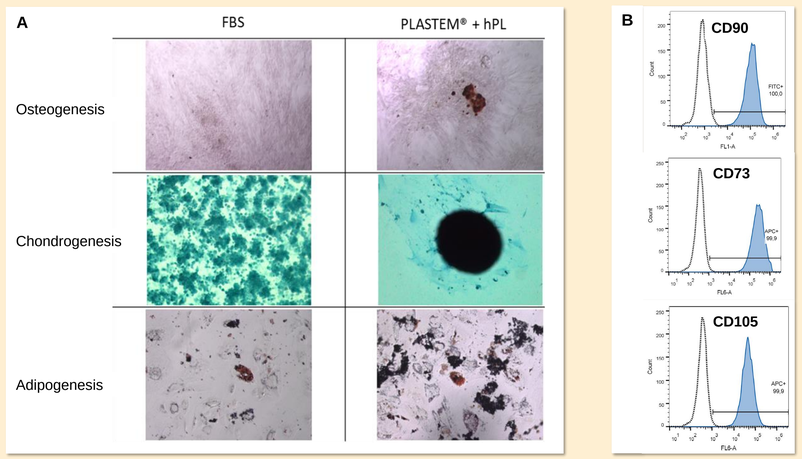

FIGURE 2. (A) Differentiation of umbilical cord human mesenchymal stromal cells (UC-hMSCs) into the osteogenic, chondrogenic and adipogenic lineages. (B) Immunophenotype flow cytometry histograms for UC-hMSCs supplemented with PLASTEM® 10% + hPL 0.5% determining the expression of CD markers CD90, CD73, and CD105.

- In **immunomodulation assays**, hMSCs supplemented with PLASTEM® 10%+ hPL 0.5% reduced PBMCs absolute proliferation percentage (31.5%,Promocell; 37.6%,Creative Bioarray) compared with PBMCs alone (82.2%) (Figure 3).

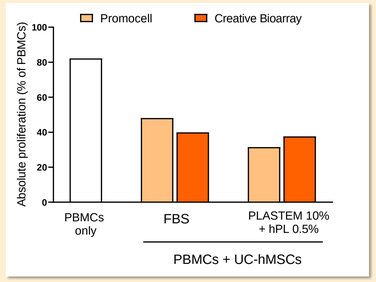

FIGURE 3. Immunomodulation assay by co-culture of PBMCs and UC-hMSCs. Absolute proliferation of PHA-activated PBMC alone or in a co-culture with UC-hMSCs from two suppliers (Promocell and Creative Bioarray) and supplemented with FBS or PLASTEM® 10% + hPL 0.5%.

- The **immunophenotype** of T cells supplemented with PLASTEM® 15% showed an increased proportion of CD4+ T helper cells compared with FBS or hSerAB (Table 1).

Table 1. Immunophenotyping of T cells cultured with different media supplements are determined by cell surface expression of CD markers

Cell population	CD Surface marker	% of T cells, range, n=2		
		FBS	hSerAB	PLASTEM 15%
T- helper cells (CD4+)	$CD45^+CD3^+CD4^+$	60.1 - 60.3	46.1 - 46.8	65.4 - 80.0
Cytotoxic T cells (CD8+)	$CD45^+CD3^+CD8^+$	9.6 - 37.3	25.4 - 52.1	3.47 - 16.1
Naïve T cells	$CD45^+CD3^+CD4^+CCR7^+CD45RA^+$	3.4 - 3.7	1.1 - 3.1	1.1 - 3.1
	$CD45^+CD3^+CD8^+CCR7^+CD45RA^+$	7.9 - 17.9	1.0 - 4.1	2.3 - 12
Effector T cells	$CD45^+CD3^+CD4^+CCR7^-CD45RA^+$	57.3 – 60	60.1 - 68.9	73.9 - 76,6
	$CD45^+CD3^+CD8^+CCR7^-CD45RA^+$	77.9 - 88.5	92.2 - 95.8	87.8 - 94.2
Effector memory T cells	$CD45^+CD3^+CD4^+CCR7^-CD45RA^-$	34.4 - 35.9	27 - 38.2	21.0 - 21.7
	$CD45^+CD3^+CD8^+CCR7^-CD45RA^-$	2.5 - 3.8	3.1 - 3.5	0.2 – 3.3
Central memory T cells	$CD45^+CD3^+CD4^+CCR7^+CD45RA^-$	1.8 - 3,6	0.7 - 1.0	1.2 - 1.3
	$CD45^+CD3^+CD8^+CCR7^+CD45RA^-$	0.3 - 1.1	0.2 - 0.2	0 - 0.4

REFERENCES

[1] Trento, C. et al. Biol. Blood Marrow Transplant. 2018;24,2365–70
[2] Blazquez-Prunera, A. et al. Stem Cell Res Ther. 2017;8(1):103

DISCLOSURES

Francisco J. Belda is a full-time employee of Grifols, a manufacturer of PLASTEM®.

CONCLUSIONS

PLASTEM® demonstrated suitability and consistency for cell therapy manufacturing process and it was proved as a competitive and robust supplement for cell culture.

The culture media supplement will help to streamline regulatory submissions, enabling the reproducible generation of potent quality-assured cell therapy.

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