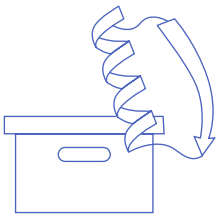
PLASTEM® - Xeno-free GMP grade hMSC Cell Culture Supplement

BioSupplies
Biological material solutions

Proteins

PLASTEM



Human mesenchymal stromal cell (hMSC) therapy has been gaining considerable interest for its immunosuppressive and regenerative potential. Production of a MSC therapeutic product requires cell culture with a supplemented media. In hMSC culture, the safety, quality and consistency of culture media supplements and other raw materials are vital for the efficacy and consistency of the final product. Supplements must be of high quality and compliant with regulatory standards, but they must have high safety standards, minimal batch variation and large batch sizes. 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. This application note describes how PLASTEM® combined with human platelet lysate (hPL) is an effective media supplement for hMSC culture.

INTRODUCTION

A number of options exist for hMSC culture media supplementation including serum-free chemically defined medias, fetal bovine serum (FBS) and human plasma-derived supplements. Serum-free chemically defined media, while advantageous, often requires pre-coating of the culture surface adding another layer of cost and complexity to the process.[1]

FBS is used as a culture medium supplement for research and clinical MSC culture,[2] despite the risk for transmission of pathogens and xeno-immunization. Guidelines on the use of bovine serum in the manufacture of human biological medicinal products (EMA/CHMP/BWP/457920/2012) emphasize that, when a manufacturer has a choice between the use of ruminant or non-ruminant materials, the use of non-ruminant material is strongly recommended.

In addition to safety concerns, there is considerable batch-to-batch variability in FBS.

In order to improve safety, overcome batch-to-batch variation and avoid animal-derived material we have introduced PLASTEM®. PLASTEM® is a consistent culture media supplement with each batch manufactured from more than 1000 plasma donations. PLASTEM® is manufactured using the same rigorous standards applied to the manufacture of Grifols therapeutic products and provides a robust safety profile. Previous studies have shown that combining PLASTEM® with recombinant growth factors produces an effective media supplement for hMSC culture.[3-5] This application note demonstrates that, when used in combination with PLASTEM®, significantly lower amounts of hPL (10% of that typically required) can support the growth of hMSCs from three separate sources.[6,7]

QUALITY

The quality standards of a human plasma-derived medicine are applied to PLASTEM® production.

CONSISTENCY

PLASTEM® is manufactured from plasma pools containing more than 1000 donations and has good batch-to batch consistency.

SAFETY

Plasma is collected from healthy donors in US-based FDA-licensed plasma centres. PLASTEM® is manufactured using the same safety standards as plasma-derived intravenous (IV) therapeutic products and includes viral removal / inactivation steps (eg, gamma irradiation treatment), and a plasma inventory hold period.

CAPACITY

Grifols has a network of more than 300 US-based plasma donation centres and can fractionate more than 13 million litres of plasma per year.

GRIFOLS

PLASTEM® + hPL SUPPORTS THE EXPANSION OF hMSCs[6]

Expansion of hMSCs in PLASTEM-supplemented media was evaluated. Culture media was supplemented with either FBS (10%) or PLASTEM® (10%) + hPL (0.5%). Cell growth was determined for a continuous 13-day experiment through a trypan blue exclusion assay, in which cell number and cell viability were assessed. PLASTEM® combined with hPL supported the growth of hMSCs from three different sources tested: Bone Marrow (BM) (Figure 1A), Adipose Tissue (AT) (Figure 1B) and Umbilical Cord (UC) (Figure 1C).

Figure 1

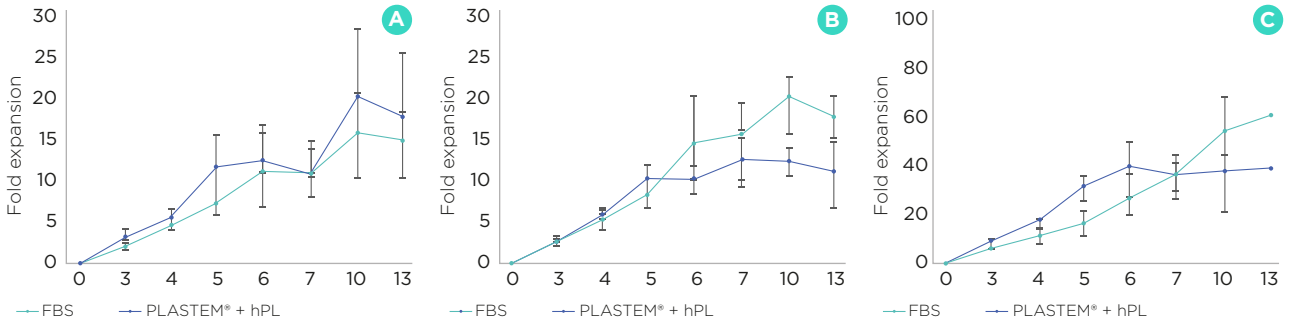


Figure 1. Determination of cell proliferation in (A) BM-MSCs, (B) AT-MSCs or (C) UC-MSCs supplemented with FBS 10% or PLASTEM® 10% + hPL 0.5%.

hMSCs CULTURED WITH PLASTEM® + hPL MAINTAIN EXPRESSION OF PHENOTYPIC CELL SURFACE MARKERS[6]

hMSCs from the three different origins cultured in media supplemented with PLASTEM® (10%) + hPL (0.5%) presented the correct hMSC phenotype (BM-MSC, AT-MSC and UC-MSC) according to the International Society for Cellular Therapy (ISCT) guidelines (Dominici et al., 2006). All three hMSC origins tested positive for CD73, CD90 and CD105, and negative for CD14, CD19, CD45, CD34 and HLA-DR (Figure 2).

Figure 2

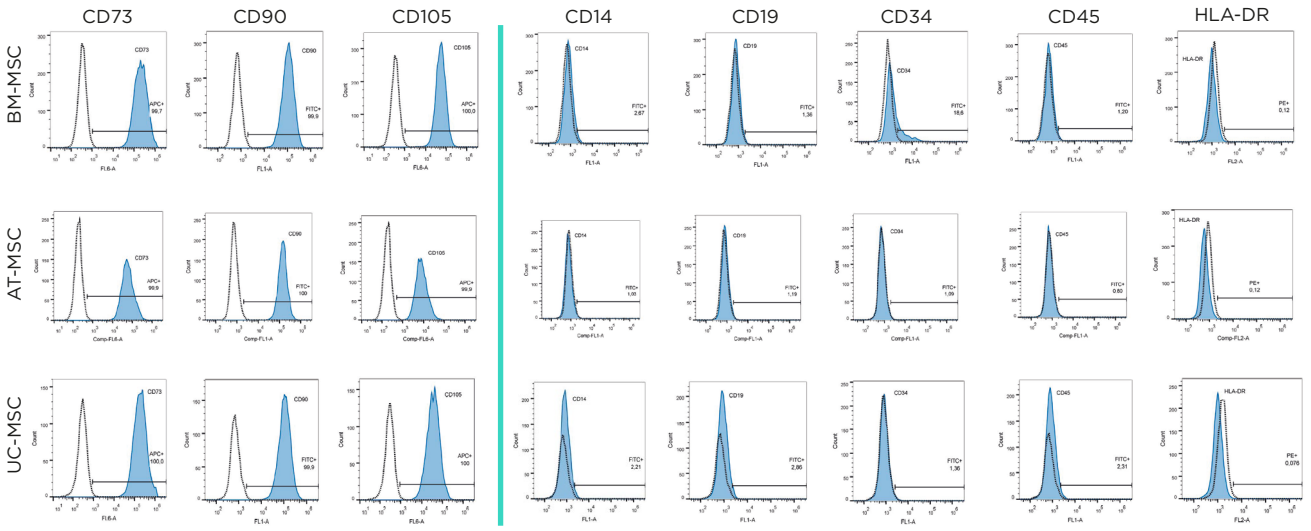


Figure 2. Phenotypic characterisation of hMSC as determined by flow cytometry. Figures are representative examples of each tested origin: Bone Marrow (BM-MSC), Adipose Tissue (AT-MSC) and Umbilical Cord (UC-MSC). Empty dotted lines correspond to the negative control, and blue histograms to the specific antibody staining.

hMSCs CULTURED WITH PLASTEM® + hPL PRESERVE THEIR MULTIPOTENTIALITY[6]

hMSCs cultured in media supplemented with PLASTEM® (10%) + hPL (0.5%) maintain the ability to differentiate into three standard cell types, namely, osteoblasts, adipocytes or chondrocytes, as demonstrated by the multipotentiality test defined by The ISCT (Dominici et al., 2006). hMSCs from the three major origins (BM-MSC, AT-MSC and UC-MSC) were expanded with the corresponding media, and then cultured with the relevant differentiation medium (Figure 3 A-C).

Figure 3

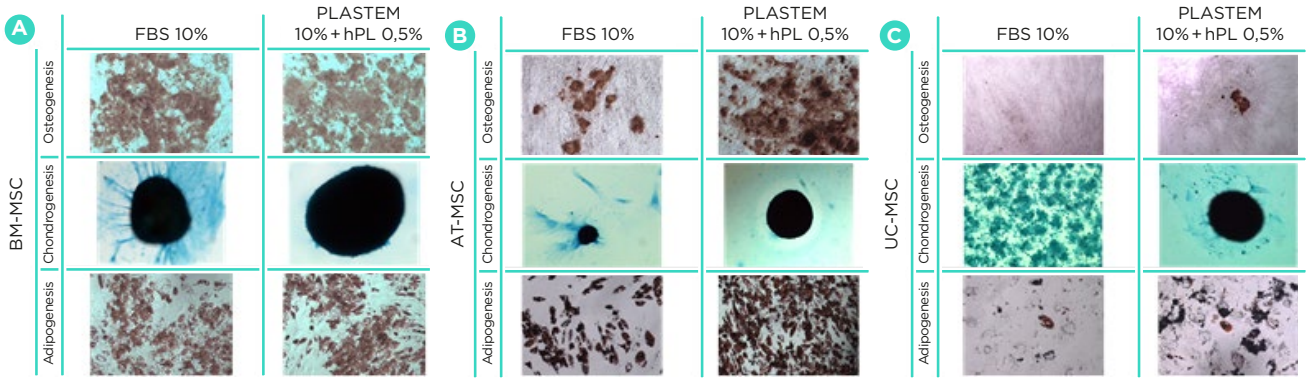


Figure 3. Differentiation of hMSCs into the osteogenic, chondrogenic and adipogenic lineages. Figures are representative examples of each tested origin: (A) Bone Marrow (BM-MSC), (B) Adipose Tissue (AT-MSC) and (C) Umbilical Cord (UC-MSC).

hMSCs CULTURED WITH PLASTEM® + hPL RETAIN THEIR IMMUNOMODULATORY PROPERTIES[6]

One of the clinical applications of approved hMSC therapies is immunomodulation. Therefore, hMSC immunomodulatory properties were assessed after their expansion with PLASTEM® (10%) + hPL (0.5%). BM-MSC, AT-MSC and UC-MSC were co-cultured with stimulated Peripheral Blood Mononuclear Cells (PBMCs). Proliferation of PBMCs was determined using a CFSE-based proliferation assay. hMSCs cultured with PLASTEM® + hPL were able to strongly suppress proliferation of stimulated PBMCs to a level higher than that observed with hMSCs cultured in media supplemented with FBS (Figure 4).

Figure 4

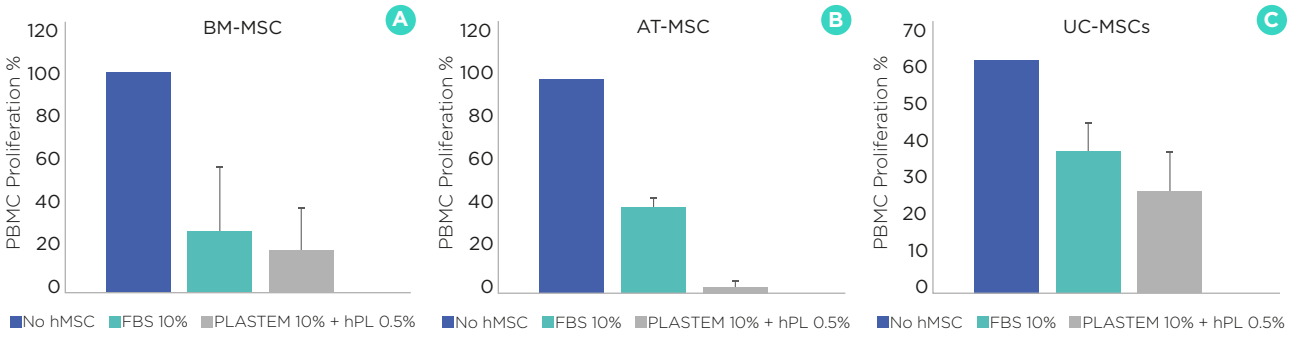


Figure 4. hMSCs (BM-MSC (A), AT-MSC (B), and UC-MSC (C)) expanded in either FBS 10% or PLASTEM® 10% + hPL 0.5% inhibited the proliferation of stimulated PBMCs when co-cultured.

PLASTEM® IN COMBINATION WITH hPL AT A 10-FOLD LOWER THAN THE STANDARD CONCENTRATION IS AN EFFECTIVE CULTURE SUPPLEMENT FOR hMSCs.[6,7]

Additional advantages to PLASTEM® (10%) + hPL (0.5%) combination:

- There is no requirement for FBS, removing the risk of xeno pathogen transmission and xeno-immunisation
- 10-fold reduction in hPL use means validation of fewer batches and less exposure to potential batch-to-batch variation
- PLASTEM® is a well-defined, GMP grade raw material produced following the same standard as Grifols therapeutic products. It also is accompanied by strong regulatory support from Grifols experts with experience in global registration of therapeutic products
- For further defined culture conditions, PLASTEM® can be combined with recombinant growth factors such as fibroblast growth factor (FGF)

PLASTEM® demonstrated suitability and consistency for the cell therapy manufacturing process.[6] The culture media supplement will help to streamline regulatory submissions, enabling the reproducible generation of potent quality-assured cell therapies.

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