PLASTEM® - GMP grade hMSC Cell Culture Supplement

BioSupplies
Biopharma
Cell Culture

The performance of PLASTEM® as a human mesenchymal stem cell (hMSCs) culture supplement was assessed according to the three International Society for Cell Therapy (ISCT) minimal criteria for defining hMSC: adherence to plastic, expression of CD markers, and the ability to differentiate into chondrocytes, adipocytes, and osteogenic cells (Dominici M, et al. 2006). Additionally, the immunodulatory properties of PLASTEM® cultured cells was also assessed. The information presented below is a combination of Grifols internal R&D data and data obtained from peer-reviewed publications.

CULTURE OF UMBILICAL CORD hMSCs

Firstly, proliferation of umbilical cord-derived hMSCs (UC-MSCs) in non-coated tissue culture plates was evaluated. Culture media was supplemented with either acronym fetal bovine serum (FBS) 10%, 10% PLASTEM® with recombinant fibroblast growth factor (FGF) or 10% PLASTEM® with 0.5% human platelet lysate (hPL). Cells were counted manually using a trypan blue assay.

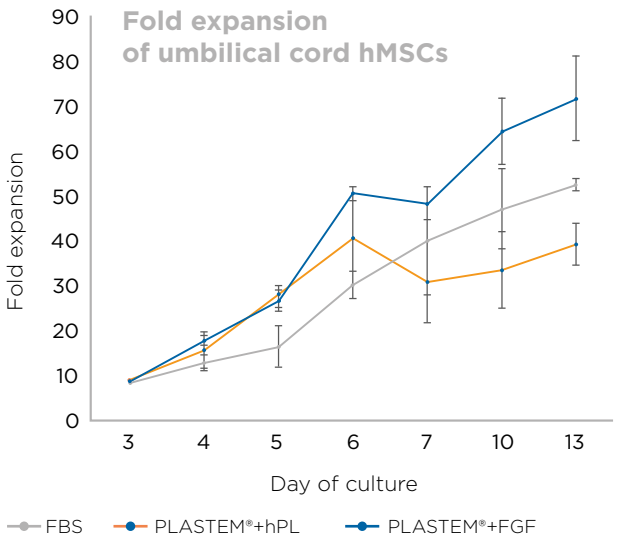
PLASTEM® combined with either FGF or hPL functioned comparably as a media supplement compared to FBS (Figure 1). Addition of small quantities of hPL or recombinant growth factors creates a reproducible, highly consistent and high-quality cell culture media.

PLASTEM® is a pharmaceutical grade, highly consistent and safe human plasma-derived cell culture supplement. PLASTEM® offers product registration advantages to customers seeking regulatory support.

hMSCs CULTURED WITH PLASTEM® MAINTAIN EXPRESSION OF PHENOTYPING CELL SURFACE MARKERS

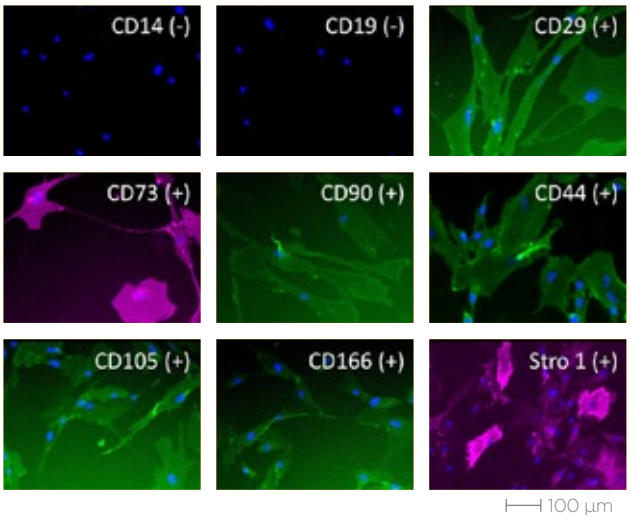
hMSCs cultured in PLASTEM® and FGF supplemented media presented the normal hMSC phenotype, being negative for CD14, CD19 and positive for CD29, CD44, CD73, CD90, CD105, CD166, and Stro-1 (Figure 2).

Figure 1



Fold expansion of umbilical cord-derived hMSCs (10% PLASTEM + 0.5% hPL, 10% PLASTEM + FGF, FBS 10%). Grifols, Data on file.

Figure 2



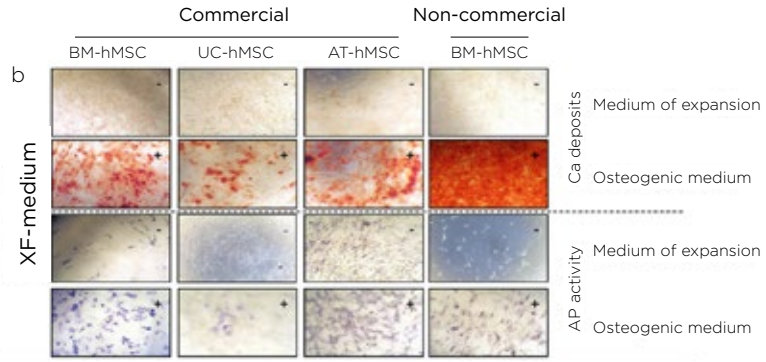
Phenotypic characterization of human mesenchymal stem cells. Expression of the typical human mesenchymal stem cells surface markers by xeno-free (PLASTEM® + FGF) expanded human mesenchymal stem cells as determined by immunofluorescence staining (representative images). Reproduced from Blázquez-Prunera A. et al. 2017.

GRIFOLS

PLASTEM® CULTURED HMSCS PRESERVE THEIR MULTIPOTENTIALITY

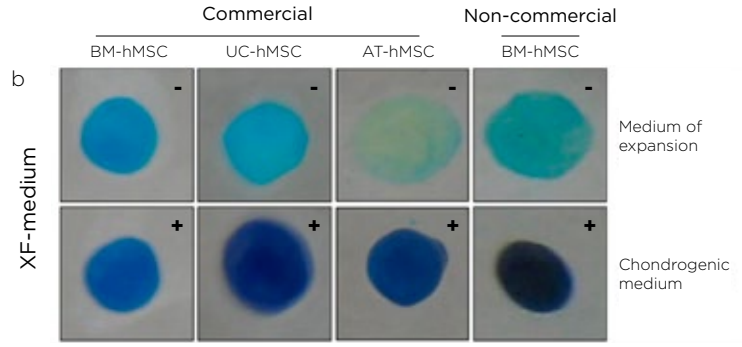
Multipotentiality is a defining characteristic of hMSCs. Blázquez-Prunera A, et al. (2017) evaluated the effect of PLASTEM® on the ability of hMSCs to differentiate. Figure 3, 4 and 5 are reproduced from Blázquez-Prunera A, et al. (2017), and demonstrate that hMSCs cultured in media supplemented with PLASTEM® and FGF retain the ability to differentiate.

Figure 3



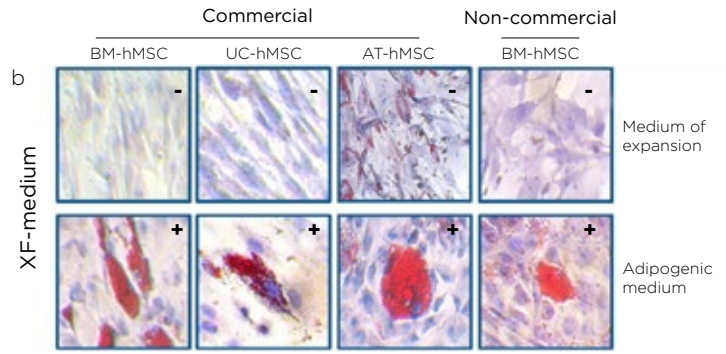
Osteogenic differentiation of human mesenchymal stem cells. Representative images of commercial and non-commercial bone marrow (BM)-, adipose tissue (AT)- and umbilical cord (UC)-derived human mesenchymal stem cells (hMSCs) expanded in xeno-free medium (XF-Medium = PLASTEM® + FGF). hMSCs were cultured for 21 days in osteogenic differentiation medium or the medium of expansion. After 7 days, the alkaline phosphatase (AP) activity was stained in blue with BCIP/NBT. After 21 days, the extracellular calcium (Ca) deposits was stained with Alizarin Red S in red. Reproduced from Blázquez-Prunera A, et al. 2017.

Figure 4



Chondrogenic differentiation of human mesenchymal stem cells. Representative images of commercial and non-commercial bone marrow (BM)-, adipose tissue (AT)- and umbilical cord (UC)- derived human mesenchymal stem cells (hMSCs) expanded in xeno-free medium (XF-Medium = PLASTEM® +FGF). hMSCs were cultured for 21 days in chondrogenic differentiation medium or the medium of expansion. Alcian Blue was used to stain the extracellular cartilage matrix in dark blue. Reproduced from Blázquez-Prunera A, et al. 2017.

Figure 5



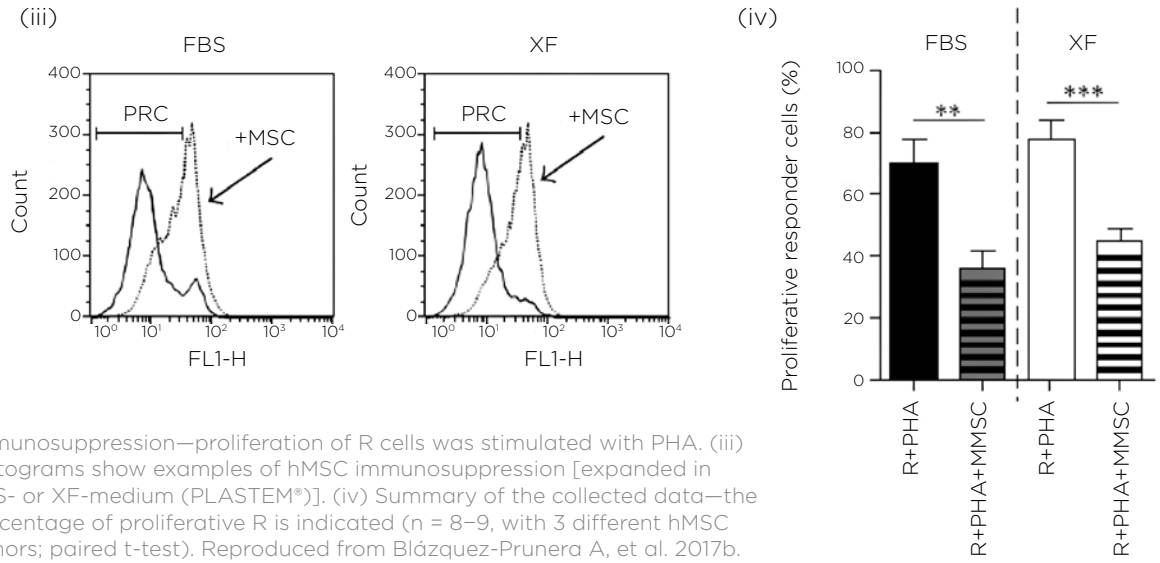
Adipogenic differentiation of human mesenchymal stem cells. Representative images of commercial and non-commercial bone marrow (BM)-, adipose tissue (AT)- and umbilical cord (UC)- derived human mesenchymal stem cells (hMSCs) expanded in xeno-free medium (XF-Medium = PLASTEM® + FGF). hMSCs were cultured for 14 days in adipogenic differentiation medium or the medium of expansion. Lipid vesicles in adipocytes were stained with Oil Red O and are seen in red. Reproduced from Blázquez-Prunera A, et al. 2017.

PLASTEM® CULTURED hMSCs RETAIN THEIR IMMUNOMODULATORY PROPERTIES

One of the primary applications of approved hMSC therapies is immunomodulation. Therefore, hMSC immunomodulation capability after culture with PLASTEM® should be assessed. Blázquez-Prunera A, et al. (2017b) found that PLASTEM® cultured hMSCs were able to suppress proliferation of mitogen-stimulated peripheral blood mononuclear cells (PBMCs) (Figure 6) and were able to stimulate proliferation of resting PBMCs, thus maintaining their immunostimulatory properties (Figure 7).

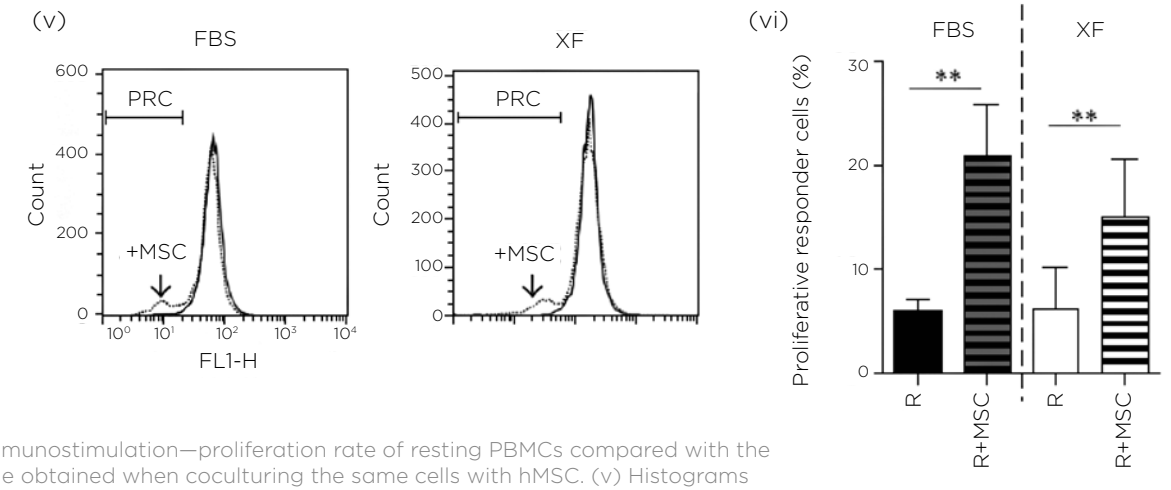
These results agree with the current view that the immunomodulatory properties of hMSC depend on the niche; in an inflammatory niche, cytokines prime hMSCs, so they can act as immunosuppressor agents, but in a niche where no proinflammatory cytokines are present, hMSCs act as immunostimulatory agents.

Figure 6



Immunosuppression—proliferation of R cells was stimulated with PHA. (iii) Histograms show examples of hMSC immunosuppression [expanded in FBS- or XF-medium (PLASTEM®)]. (iv) Summary of the collected data—the percentage of proliferative R is indicated (n = 8–9, with 3 different hMSC donors; paired t-test). Reproduced from Blázquez-Prunera A, et al. 2017b.

Figure 7



Immunostimulation—proliferation rate of resting PBMCs compared with the one obtained when coculturing the same cells with hMSC. (v) Histograms of these two conditions with hMSC expanded in FBS-Medium or XF-Medium (PLASTEM®). (vi) Summary of the collected data—the percentage of proliferative R is indicated (n = 8, with 3 different hMSC donors, paired t-test). Reproduced from Blázquez-Prunera A, et al. 2017b.

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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 (e.g. Gamma irradiation treatment), and plasma inventory hold period.

CAPACITY

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

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