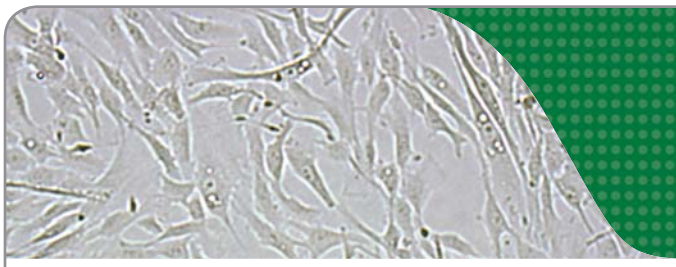

TECHNICAL BULLETIN

Characterization of Human MSCs Cultured in MesenCult™-XF

Introduction

Mesenchymal stem cells (MSCs), which have been more recently termed multipotent mesenchymal stromal cells, are plastic-adherent, fibroblast-like cells, which in culture are able to self-renew and differentiate into bone, adipose and cartilage tissue.[1,2] They can be isolated from bone marrow, adipose tissue and a variety of other sources. Accumulating evidence indicates a perivascular location for these cells, leading to the suggestion that MSCs are pericytes which exist in intimate contact with endothelial cells in capillaries and microvessels in multiple organs.[3-10]

Given the ability of MSCs to give rise to multiple tissue types, as well as their efficacy in the modulation of immune cells, there is considerable interest in utilizing MSCs in a broad repertoire of cell-based therapies.[11-14] To obtain sufficient numbers of cells for research and therapeutic applications, MSCs must be expanded in vitro.[14] However, traditional media for expanding MSCs are serum-based and therefore exhibit inherent variability, which can lead to inconsistent cell purity and potency. Furthermore, the presence of non-human, animal-derived proteins in the expansion medium raises concern when the MSCs are to be used in clinical applications.[15]

To facilitate mesenchymal stem cell research, STEMCELL Technologies has developed a xeno-free, serum-free medium (MesenCult™-XF) which promotes superior clonogenic growth and supports long-term expansion of cells while maintaining multi-lineage differentiation potential. Here we describe the characterization of human bone marrow (BM)-derived MSCs cultured in MesenCult™-XF.

Materials and Methods

Culture

Refer to the Technical Manual: Culture of Human Mesenchymal Stem Cells Using MesenCult™-XF Medium (Manual Catalog #29184), available at www.stemcell.com, for additional details on the culture procedure.

Mononuclear cell (MNC) suspensions from fresh human bone marrow (BM) were prepared as indicated in the MesenCult™-XF Technical Manual and cultured in either MesenCult™-XF (Catalog #05420) or a fetal bovine serum (FBS)-based medium. For cells cultured in MesenCult™-XF, tissue culture plates were coated with MesenCult™-SF Attachment Substrate (Catalog #05424).

CFU-F Assay

Clonogenic growth was analyzed by low density plating of BM MNCs. For MesenCult™-XF, cells were plated at a density of 1.5 - 5 x 10^4 MNCs/cm^2 and for FBS-based medium, cells were plated at a density of 2 - 10 x 10^4 MNCs/cm^2. CFU-F were fixed with methanol, stained with Giemsa and enumerated with the aid of a microscope. Due to the observed increased proliferative capacity of CFU-F forming cells cultured in MesenCult™-XF, CFU-F were fixed and stained 10 days post plating in MesenCult™-XF and 14 days post plating in FBS-based medium.

Expansion Analysis

When seeding MSCs from primary BM, 3.0 - 7.0 x 10^4 MNCs/cm^2 were plated in MesenCult™-XF or 3.0 - 7.0 x 10^5 MNCs/cm^2 were plated in FBS-based medium. Cultures were passaged when they reached 80% confluence. The MesenCult™-ACF Dissociation Kit (Catalog #05426) was used for passaging cells in MesenCult™-XF, whereas Trypsin/EDTA (0.25%) was used to passage cells cultured in FBS-based medium. When subculturing the cells (i.e. seeding cells at passage 1 and greater), between 1.5 - 4 x 10^3 cells/cm^2 were plated in MesenCult™-XF and between 4 - 10 x 10^4 cells/cm^2 were plated in FBS-based medium.

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Phenotype Analysis

MSCs cultured in MesenCult™-XF or FBS-based medium were harvested, washed and labeled with fluorescent-conjugated (PE, FITC or APC) antibodies to CD105, CD90, CD73, CD45, CD11b and CD34. Flow cytometry analysis was performed using the BD FACSCalibur™.

Differentiation Analysis

The multi-lineage differentiation potential of MSCs cultured in MesenCult™-XF was compared to MSCs cultured in FBS-based medium. At the specified passage number, the expansion medium was replaced with the appropriate differentiation medium. MesenCult™ Adipogenic Stimulatory Supplement (Human; Catalog #05403) was used to induce adipogenic differentiation of passage 1 (P1) MSCs. Adipocytes were detected by staining cultures with Oil Red O. MesenCult™ Osteogenic Kits (Catalog #05434 (MesenCult™-XF) or Catalog #05404 (FBS-based medium)) were used to differentiate P4 MSCs along the osteogenic lineage. After mineralization was observed, cultures were stained with Alizarin Red to detect calcium deposits, which are indicative of bone formation. To induce chondrocyte differentiation, 5 x 10^5 P2-P8 MSCs were centrifuged to form micromass pellets in conical tubes and cultured in chondrocyte differentiation medium for 3 weeks. The pellet was subsequently fixed, embedded in paraffin, sectioned and stained with Alcian Blue or processed to detect Collagen Type II.

Immunosuppression Assay

Passage 2 MSCs generated in MesenCult™-XF or FBS-based medium were treated with 25 µg/mL mitomycin C for 30 minutes at 37°C prior to co-culture with T cells. Human T cells were isolated from previously frozen PBMCs using EasySep™ (Catalog #19051) immunomagnetic separation and fluorescently labeled with 1 µM 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (5(6)CFDA,SE; CFSE). CFSE-labeled T cells (2 x 10^5 cells) were co-cultured with MSCs (1 x 10^5 cells) in serum-free medium supplemented with 100 U/mL IL-2. T cells were stimulated with tetrameric antibody complexes against CD3ε, CD28 and CD2. On days 3 and 7, cells were harvested, stained with a fluorochrome conjugated anti-CD45 antibody and propidium iodide and the T cell division history measured as CFSE dye dilution analyzed by flow cytometry.

Results

MesenCult™-XF Enables Superior Clonogenic Growth of MSCs Compared to FBS-Based Medium

To assess clonogenic growth of MSCs in MesenCult™-XF compared to FBS-based medium, the frequency of colony-forming unit - fibroblast (CFU-F) was assessed. CFU-F frequencies (119 ± 33 and 109 ± 16 per 10^6 cells plated (Table 1)) were comparable, however, colonies generated in MesenCult™-XF were twice as large as those obtained in FBS-based medium (average diameter 5.7 ± 0.3 mm vs 2.8 ± 0.99 mm).

MSCs Cultured in Mesencult™-XF Expand Faster Than Cells Cultured in FBS-Based Medium

Expansion of primary BM-derived MSCs cultured in MesenCult™-XF was compared to cells cultured in FBS-based medium over 9 passages. Cell expansion was significantly higher when MSCs were cultured in MesenCult™-XF compared to FBS-based medium (Figure 1).

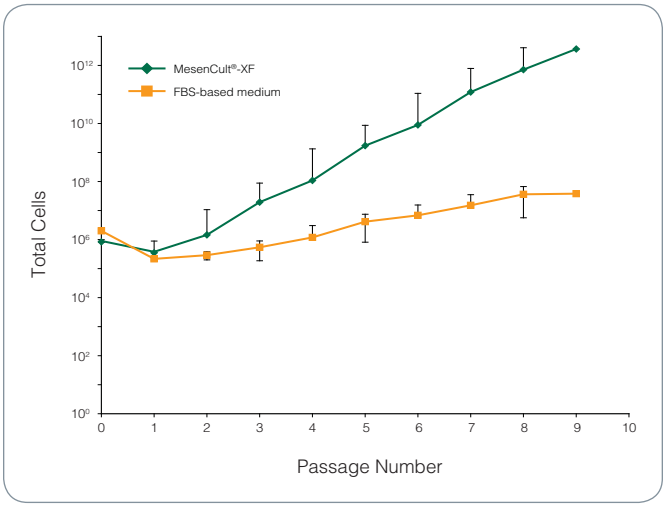

Figure 1. MSCs expand faster in MesenCult™-XF than in FBS-based medium

	CFU-F/106 BM MNCS (MEAN ± SD; N = 6)	CFU-F SIZE (MM) (AVERAGE DIAMETER ± SD; N = 3)	CFU-F SIZE (MM) (RANGE)
MesenCult™-XF	119 ± 33	5.7 ± 0.3	2.3 - 11
FBS-Based Medium	109 ± 16	2.8 ± 0.99	1.5 - 6

Table 1. Frequency and size of CFU-F generated in MesenCult™-XF or FBS-based cultures

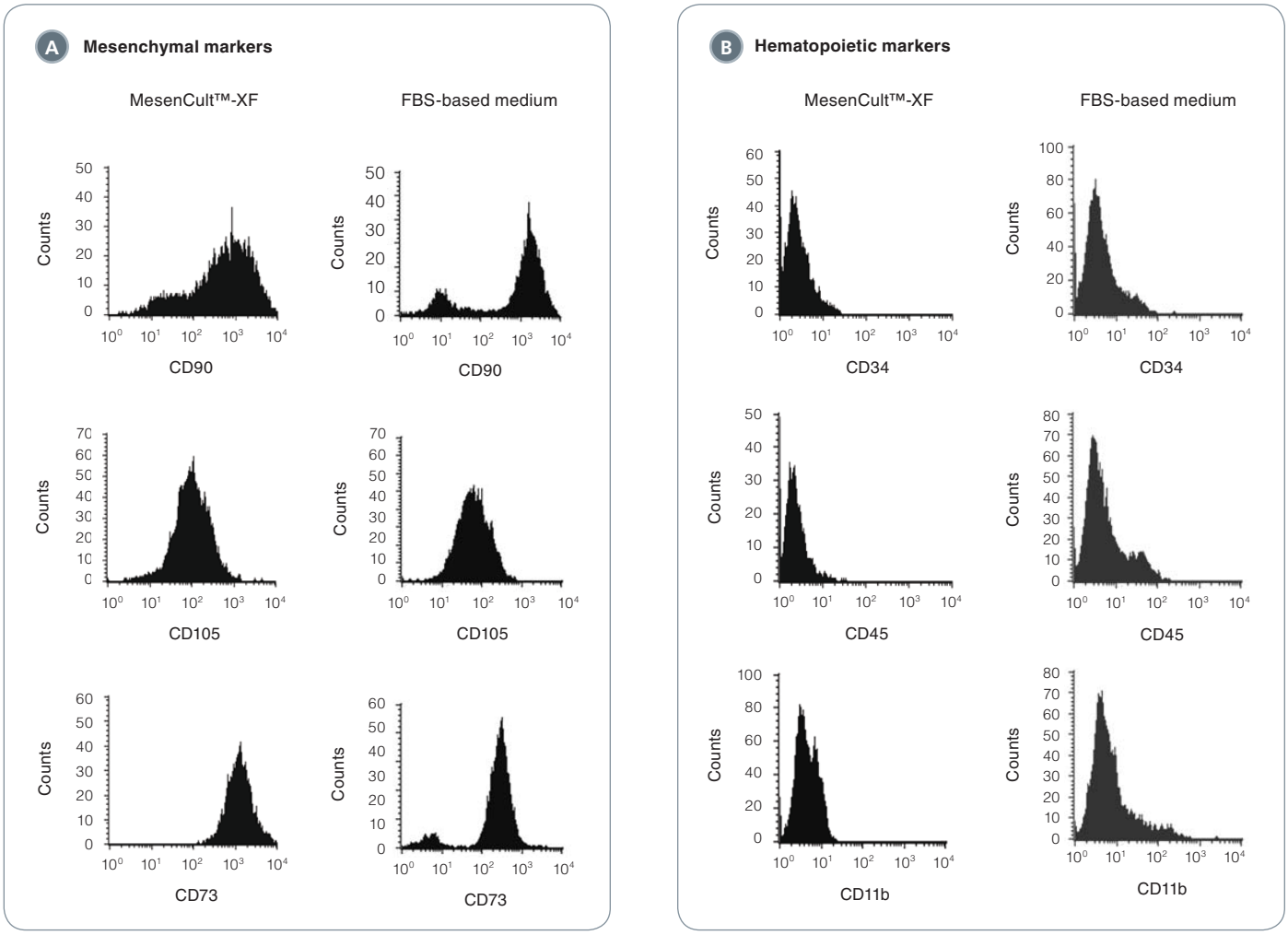

Figure 2. Phenotype of MSCs cultured for 2 passages in either MesenCult™-XF or FBS-based medium

Cells Cultured in MesenCult™-XF Express Mesenchymal Markers and Lack Expression of Hematopoietic Markers

The phenotype of MSCs cultured for 2 passages in MesenCult™-XF or FBS-based medium was analyzed by flow cytometry. The mesenchymal markers CD90, CD105 and CD73 were robustly expressed by MSCs cultured in both media (Figure 2). MSCs expanded in MesenCult™-XF showed no detectable contamination with hematopoietic cells as shown by a lack of expression of hematopoietic lineage markers CD34, CD45 and CD11b. In contrast, cultures expanded in FBS-based medium contained subpopulations of cells expressing CD45 and CD11b.

The morphology of the contaminating hematopoietic cells present in the MSC cultures resembled that of macrophages. To verify their identity, the cultures were co-labeled with CD45 and CD11b (a macrophage marker) at passage 1 (P1) and 3 (P3). The percentage of double positive cells ($CD45^+$ and $CD11b^+$) in MesenCult™-XF cultures at P1 was 1%, indicating a low level of macrophage contamination which completely disappeared by P3. In cultures expanded in FBS-based medium, macrophages were present at much higher levels, with 69% of cells double positive for CD45 and CD11b at P1 and 8% double positive cells at P3. In accordance with these observations, analysis of CD73 and CD34 expression revealed that 99% and 100% of MesenCult™-XF expanded cells were $CD73^+$ at P1 and P3, respectively, whereas a significant proportion of the cells expanded in FBS-based medium lacked expression of CD73 at both P1 and P3 (Figure 3).

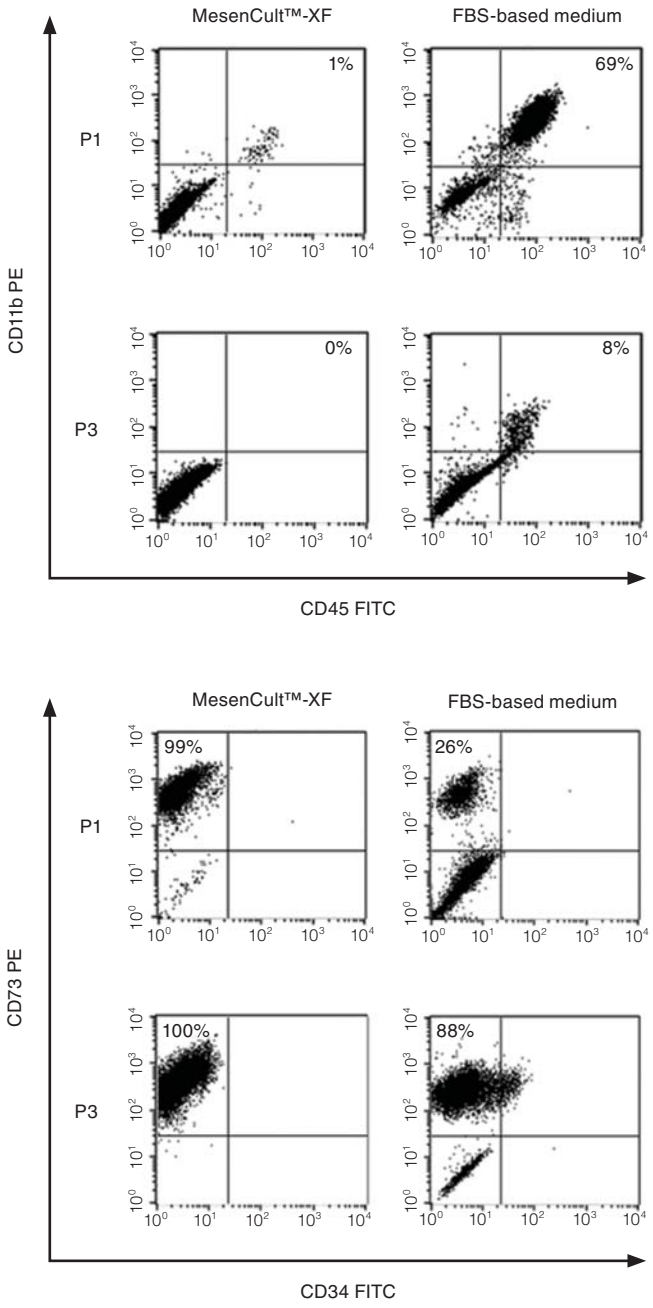

Figure 3. Phenotype of MSCs cultured for 1 (P1) or 3 (P3) passages in either MesenCult™-XF or FBS-based medium

MSCs Cultured in MesenCult™-XF Maintain Multi-Lineage Differentiation Potential

MSCs cultured in MesenCult™-XF have the ability to differentiate into adipogenic (A), osteogenic (B) and chondrogenic lineages (C, D) (Figure 4). Interestingly, chondrogenic differentiation potential of MSCs cultured in MesenCult™-XF is enhanced at early passages (e.g. P2) compared to MSCs cultured in FBS-based medium, as indicated by the larger micromass pellet and darker collagen type II staining (Figure 5A/B). Chondrogenic differentiation potential is also better maintained after multiple passages (e.g. P4, P8) when MSCs are cultured in MesenCult™-XF compared to FBS-based medium (Figure 5C-F).

MSCs Cultured in MesenCult™-XF Suppress T Cell Proliferation and Reduce Cell Cycle Division

MSCs are known to have an immunomodulatory function and have previously been shown to suppress T cell proliferation in vitro.[16-22] The immunomodulatory capacity of MSCs cultured in MesenCult™-XF was compared to MSCs cultured in FBS-based medium through co-culture of activated T cells (induced by anti-CD3 stimulation ; Figure 6). On day 3 of co-culture, MesenCult™-XF-cultured MSCs completely suppressed T-cell proliferation (i.e. the percent of proliferating T cells was equivalent to the unstimulated T cell control) whereas MSCs expanded in FBS-based medium suppressed T cell proliferation by approximately 50% (i.e. from 37% to 18%). After 7 days of co-culture, both the total number of T cells proliferating and the number of T cell division cycles were reduced in the T cell:MSC co-cultures containing MSCs previously cultured in MesenCult™-XF compared to co-cultures containing MSCs previously cultured in FBS-based medium. This indicates that MSCs cultured in MesenCult™-XF are able to more robustly suppress T cell proliferation and reduce cell cycle division than MSCs expanded in FBS-based medium.

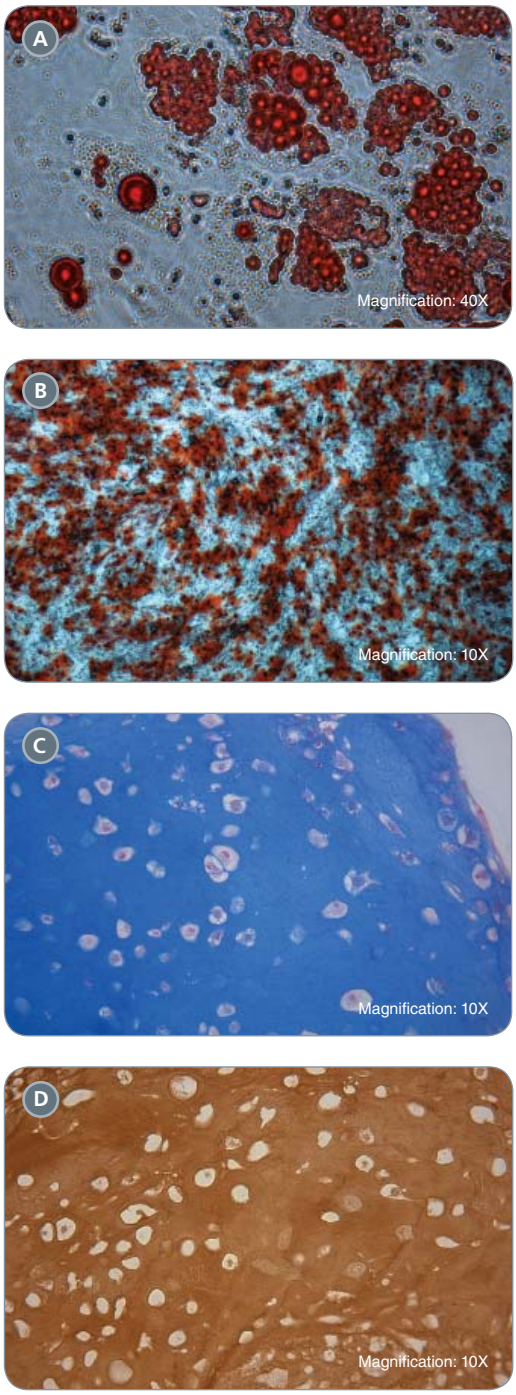

Figure 4. MSCs cultured in MesenCult™-XF maintain the ability to differentiate to adipogenic (A), osteogenic (B) and chondrogenic (C,D) lineages

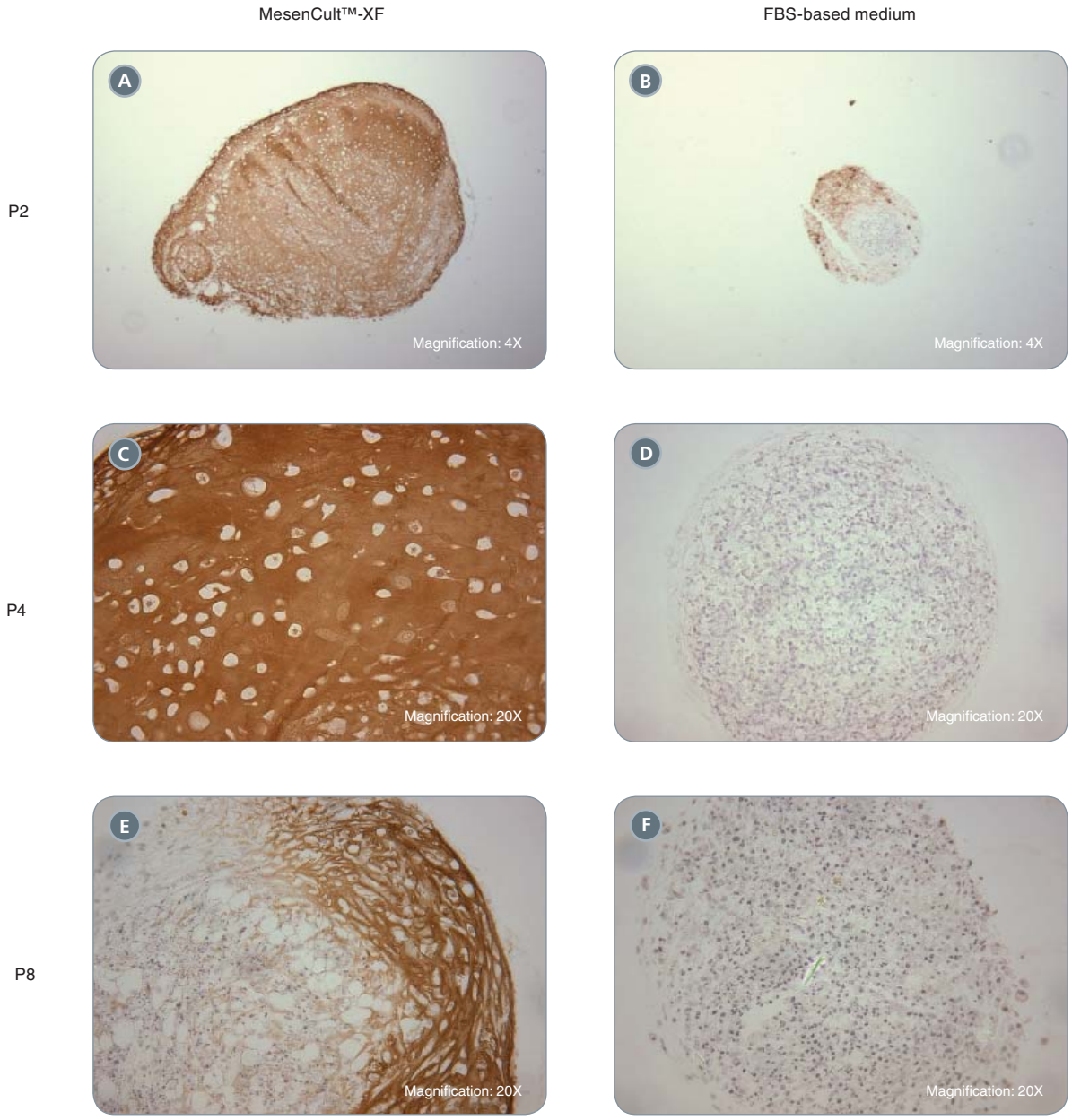

Figure 5. MesenCult™-XF enhances and better maintains chondrogenic potential (indicated by Collagen Type II staining) of cultured MSCs than FBS-based medium

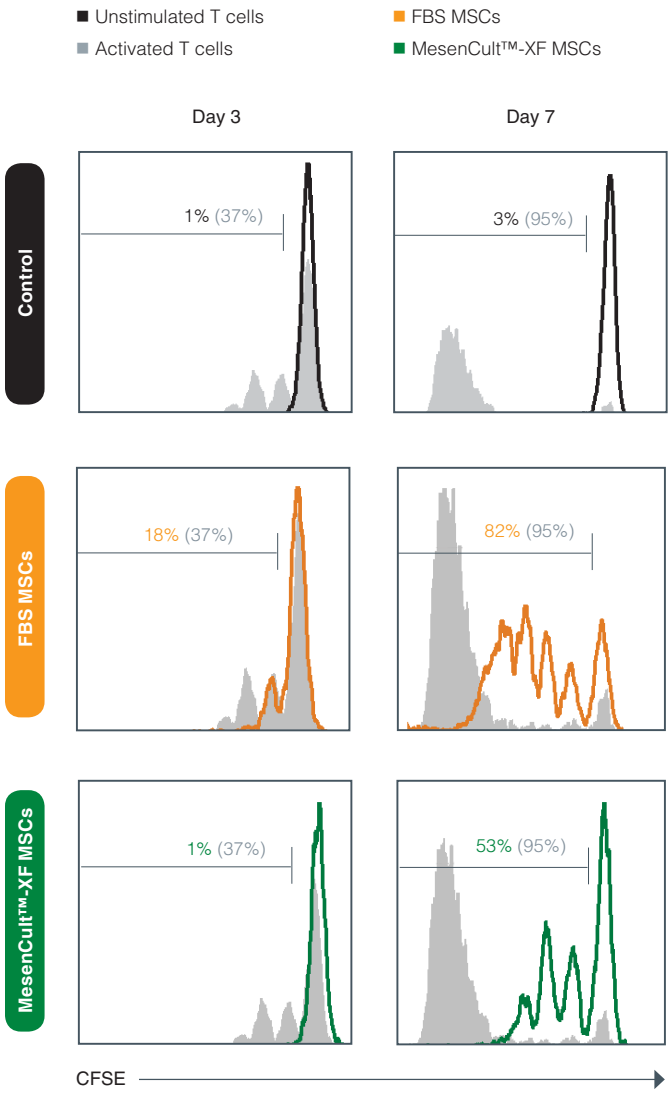

Figure 6. MSCs cultured in MesenCult™-XF suppress T cell proliferation and reduce cell cycle division more robustly than MSCs cultured in FBS-based medium

Summary

MesenCult™-XF is a xeno-free, serum-free medium for the culture of human MSCs. When compared to a FBS-based medium, MesenCult™-XF:

- Enables superior clonogenic growth of MSCs obtained from primary human bone marrow
- MSCs expand faster
- Cultured cells are $CD90^+$, $CD105^+$, $CD73^+$, $CD34^-$, $CD11b^-$ and $CD45^-$
- Cultures exhibit significantly less hematopoietic cell contamination at early passages
- Maintains multi-lineage differentiation potential
- Cultured cells exhibit superior chondrogenic differentiation potential
- Cultured MSCs suppress T cell proliferation and reduce cell cycle division more robustly

Product Information

PRODUCT	QUANTITY	CATALOG #
MesenCult™-SF Culture Kit*	1 kit	05429
MesenCult™-XF Medium§	500 mL	05420
MesenCult™-SF Attachment Substrate	5 mg	05424
MesenCult™-ACF Dissociation Kit	1 kit	05426

* MesenCult™-SF Culture Kit is comprised of MesenCult™-XF Medium (Catalog#05420) and MesenCult™-SF Attachment Substrate (Catalog #05424).

§ MesenCult™-XF Medium must be supplemented with L-Glutamine (e.g. Catalog #07100).

Characterization of Human MSCs Cultured in MesenCult™-XF

MesenCult™-XF is manufactured and controlled under a Quality Management System certified to ISO 13485 standards. MesenCult™-XF is classified as a Research Use Only (RUO) product and is not approved or intended for use in humans or animals.

Your regulatory authority will provide guidance on the requirements for ancillary reagents for cell therapy applications. Depending on the requirements, STEMCELL may be able to assist you in meeting your regulatory and quality requirements. STEMCELL stands behind the quality of our products. We welcome onsite audits of our manufacturing facilities to ensure that your quality requirements are met. If you have any questions or would like to discuss the potential use of this product for your application please contact us.

References

1. Horwitz EM, et al., Cytotherapy 7: 393-395, 2005
2. Dominici M, et al., Cytotherapy 8: 315-317, 2006
3. Caplan AI, Cell Stem Cell 3: 229-230, 2008
4. Andreeva ER, et al., Tissue Cell 30: 127-135, 1998
5. Doherty MJ, et al., J Bone Miner Res 13: 828-838, 1998
6. Bianco P, et al., Stem Cells 19: 180-192, 2001
7. Zannettino AC, et al., J Cell Physiol 214: 413-421, 2008
8. Shi S, et al., Bone Miner Res 18: 696-704, 2003
9. Sacchetti B, et al., Cell.131: 324-336, 2007
10. Crisan M, et al., Cell Stem Cell 3: 301-313, 2008
11. Horwitz EM, et al., Cytotherapy 10: 771-774, 2008
12. Jones BJ, et al., Exp Hematol 36: 733-741, 2008
13. Uccelli A, et al., Nat Rev Immunol 8: 726-736, 2008
14. Caplan AI, J Cell Physiol 213: 341-347, 2007
15. Thirumala S, et al., Expert Opin Biol Ther 13(5): 673-691, 201
16. Di Nicola M, et al., Blood 99: 3838-3843, 2002
17. Bartholomew A, et al., Exp Hematol 30: 42-48, 2002
18. Tse WT, et al., Transplantation 75: 389-397, 2003
19. Le Blanc K, et al., Scand J Immunol 57: 11-20, 2003
20. Aggarwal S and Pittenger MF, Blood 105: 1815-1822, 2005
21. Glennie S, et al., Blood 105: 2821-2827, 2005
22. Hoogduijn MJ, et al., Stem Cells Dev 16: 597-604, 2007