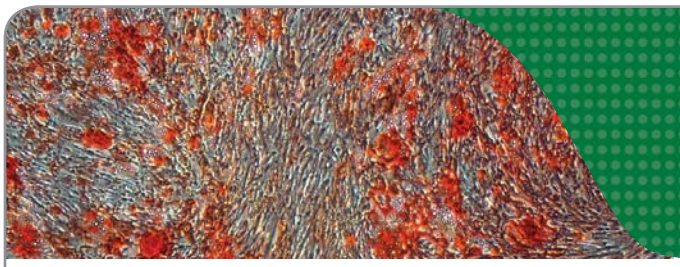


Culture Adipose-Derived Mesenchymal Stem Cells

In Serum-Free, Xeno-Free MesenCult™-XF

Introduction

Mesenchymal stem cells (MSCs) are under active investigation in tissue engineering and cell therapy research. Traditionally, MSCs were isolated from bone marrow (BM) and cultured in medium containing fetal bovine serum. This poses particular challenges to the use of human MSCs for therapeutic applications, as serum-based culture methods introduce contamination and variability concerns, MSCs occur at low frequency in bone marrow, and bone marrow donation is a slow and painful process.

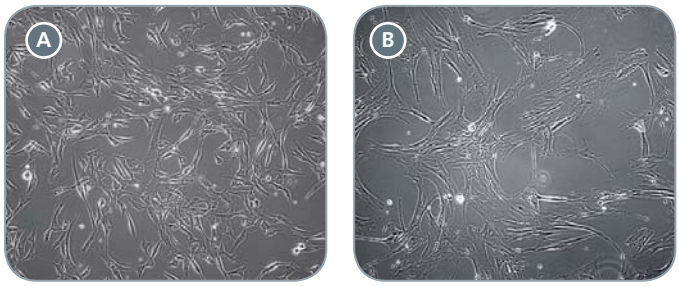
The ability to derive MSCs from more practical sources and culture them in serum-free environments would therefore address several stumbling blocks in translational MSC research. MSCs have previously been isolated from adipose tissue (Zuk et al., 2001), which is abundant and relatively easy to procure. Adipose tissue would thus be a more practical source for harvesting MSCs.

This technical bulletin presents protocols and data on a serum- and xeno-free medium (MesenCult™-XF) for the ex vivo expansion of MSCs isolated from adipose tissue (Figure 1). The data indicate that adipose tissue-derived MSCs (AT-MSCs) expand much more extensively in MesenCult™-XF as compared to a traditional serum-based medium (Figure 2), while maintaining MSC cell surface phenotypes (Figure 3) and multi-lineage differentiation potential (Figure 4). Culturing AT-MSCs with serum-free, xeno-free MesenCult™-XF Medium provides a convenient method of obtaining MSCs for translational research.

Culture adipose tissue-derived MSCs in serum-free, xeno-free MesenCult™-XF to readily obtain functional MSCs for translational research.

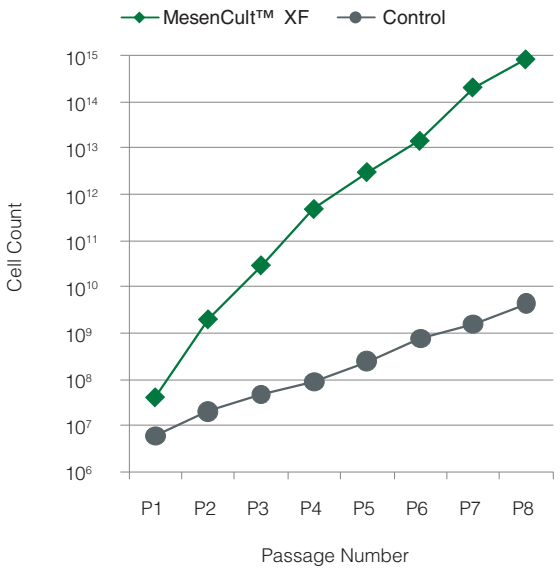
Results

FIGURE 1. Morphology of AT-MSCs Cultured in MesenCult™-XF or Control Medium



AT-MSCs cultured in MesenCult™-XF (A) for 2 passages are smaller and more spindle-shaped, compared to MSCs cultured in the serum-containing Control medium (B).

FIGURE 2. 6-Fold Higher Expansion of AT-MSCs Cultured in MesenCult™-XF Compared to Control Medium



AT-MSCs cultured for 8 passages in MesenCult™-XF showed an average fold expansion of 20.6 ± 9.8 (Mean ± SD ; n=3), at each subculture. In comparison, the proliferation of cells cultured in Control medium was about 6-fold lower with an average fold expansion of 3.12 ± 0.4 (n=4).

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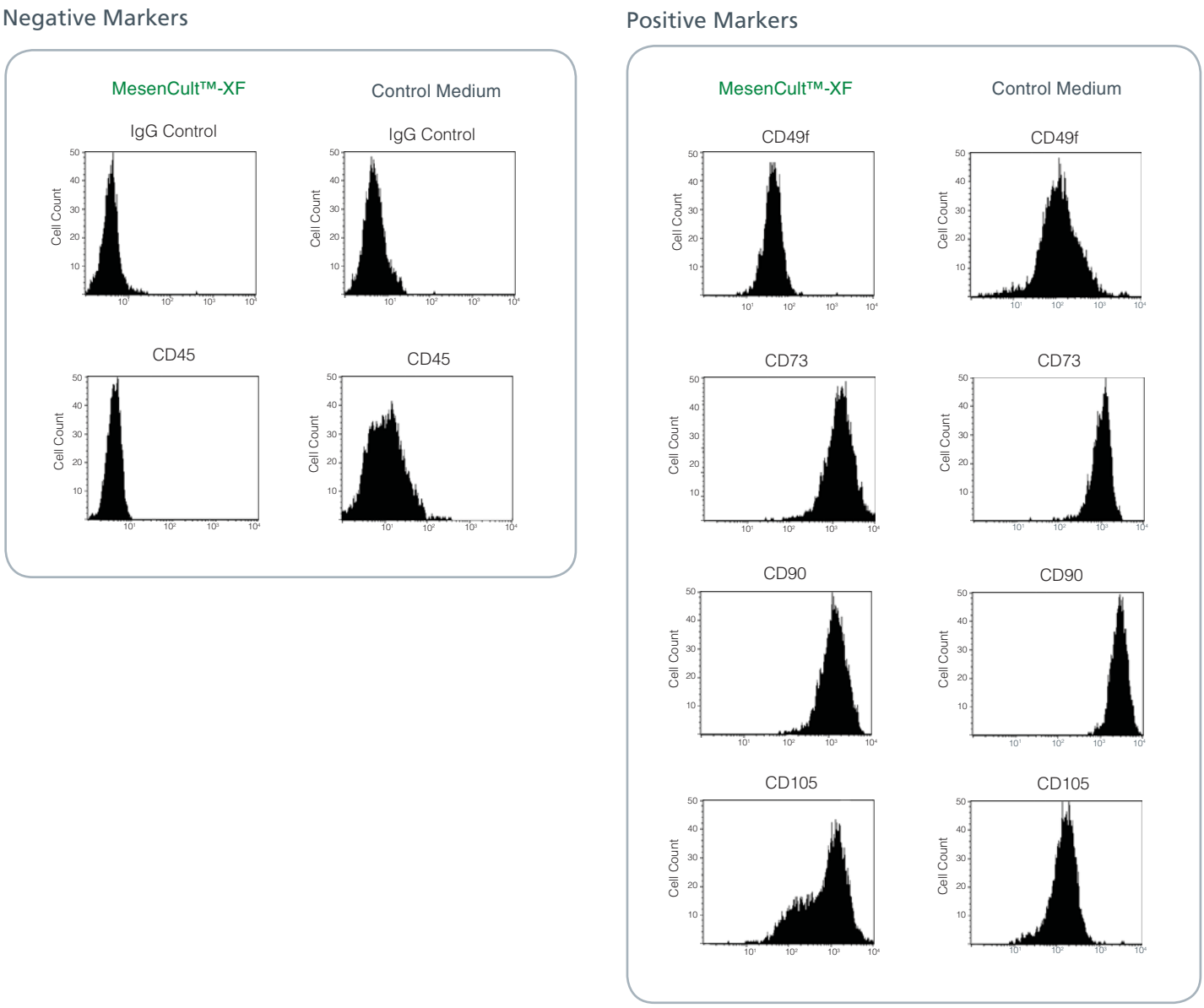
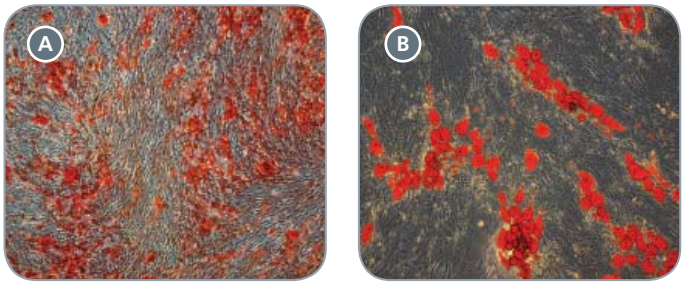
FIGURE 3. Cell Surface Marker Phenotypes of AT-MSCs Cultured in MesenCult™-XF or Control Medium



AT-MSCs cultured in either MesenCult™-XF or Control media were stained at passage 2 (P2) with antibodies to hematopoietic (CD45) or mesenchymal markers (CD49f, CD73, CD90, CD105). The cell surface phenotype of cultured AT-MSCs were similar in both media, with most cells expressing high levels of all mesenchymal antigens and low levels of hematopoietic markers (Dominici et al. 2006).

FIGURE 4. AT-MSCs Cultured in MesenCult™-XF Exhibit Robust Multi-Lineage Differentiation Potential



Passage 2 AT-MSCs readily differentiated into osteogenic (A) and adipogenic (B) lineages. The osteogenic cultures were stained with Alizarin Red to detect calcium deposition, while the adipogenic cultures were stained with Oil-Red O to detect lipids.

AT-MSC Protocols

Sample Preparation

Adipose tissue (AT) samples were obtained from reduction mammoplasty and prepared for culture using the protocol below:

1. Finely mince tissue with a scalpel.
2. Digest minced tissue in 5 mL of 0.25% Collagenase Type I per cm^3 of tissue for 60 minutes at 37°C in a shaking water bath.
3. Following digestion, place the samples upright for 5 minutes, to allow separation of the lipid layer from the aqueous layer.
4. Remove the lipid and adipose cells with a pipette or aspirator.
5. Wash the remaining cells by bringing the total volume in the tube to 50 mL with Buffer (PBS with 2% FBS + 1 mM EDTA).
6. Filter the cells through a 70 µm cell strainer to remove cell aggregates and connective tissue debris.
7. Centrifuge the cells at 400 x *g* (1200 rpm) for 10 minutes.
8. Remove the supernatant and resuspend the cells in 1 - 2 mL Buffer.
9. Dilute cells 1/50 in 3% Acetic Acid with Methylene Blue and count the total number of nucleated cells using a hemacytometer.

Cell Culture

MSCs were cultured either in serum-free, xeno-free medium (MesenCult™-XF) or in a traditional serum-containing medium (Control), using the protocol below:

1. **Coating:** For cells cultured in MesenCult™-XF, 6-well culture plates were first coated with MesenCult™-SF Attachment Substrate (required for cell adherence under serum-free culture conditions). Uncoated tissue culture treated plates were used for the Control cultures.
2. **Plating:** Primary cells were plated at 0.5 - 1.0 x 10^3 cells/cm^2 in MesenCult™-XF, and at 2.5 - 5 x 10^3 cells/cm^2 in Control medium, in a volume of 2 mL per well. It is important to plate a range of cell densities when starting with primary tissue, as variability is inherent within each sample.
3. **Passaging:** At each passage, MesenCult™-XF cultures were dissociated with MesenCult™-ACF Dissociation Kit, while Control cultures were dissociated with Trypsin-EDTA.
4. **Subculturing:** Following dissociation, cultured AT-MSCs were re-plated at 1.5 - 4 x 10^3 cells/cm^2 in MesenCult™-XF, and at 0.5 - 1 x 10^4 cells/cm^2 in Control medium.

Cell Assays

1. **Expansion:** MSCs were cultured as described above. To determine the number of cells at each passage, viable cells were counted by taking an aliquot of the harvested single cell suspension and using the Trypan Blue exclusion assay on a hemacytometer.
2. **Cell Surface Phenotype:** Passage 2 AT-MSCs cultured in MesenCult™-XF or Control 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™.
3. **Differentiation:** Passage 2 AT-MSCs cultured in MesenCult™-XF were differentiated to the osteogenic lineage using the MesenCult™ Osteogenic Stimulatory Kit (for MSCs cultured in MesenCult™-XF), and to the adipogenic lineage using the MesenCult™ Adipogenic Stimulatory Supplement (Human). The differentiation procedures are detailed in the Technical Manual: Culture of Human Mesenchymal Stem Cells Using MesenCult™-XF Medium (Manual Catalog #29184; available at www.stemcell.com).

Culture Adipose-Derived Mesenchymal Stem Cells In Serum-Free, Xeno-Free MesenCult™-XF

Product Listing

USE	PRODUCT	CATALOG #
Sample Preparation	Trypsin-EDTA (0.25%)	07901
	Collagenase Type I (0.25%)	07902
Passaging	MesenCult™-ACF Dissociation Kit	05426
	3% Acetic Acid with Methylene Blue	07060
	Trypan Blue	07050
Expansion	MesenCult™-XF Culture Medium	05420
	MesenCult™-SF Attachment Substrate	05424
Differentiation	MesenCult™ Osteogenic Stimulatory Kit (for MSCs cultured in MesenCult™-XF)	05434
	MesenCult™ Adipogenic Stimulatory Supplement (Human)	05403

References

1. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng, 7: 211-228, 2001
2. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4): 315-317, 2006.

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Webinar:
Characterization of MSCs cultured in xeno-free MesenCult™-XF
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