Enhanced Chondrogenic Potential and Immunosuppressive Activity of Human Mesenchymal Progenitor Cells Cultured in a Novel Xeno-Free Culture Medium

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Introduction

Human Mesenchymal progenitor cells (MPCs) are an important cellular source for cell therapy. MPCs are typically cultured in medium containing fetal bovine serum, which is problematic when the cells are to be utilized in clinical applications. We have developed a humanized, xeno-free medium (MesenCult®-XF) for the expansion of human MPCs and studied the proliferation, differentiation, and immunosuppressive potential of MPCs generated in this medium.

Materials & Methods

- MPCs were cultured either in xeno-free medium (MesenCult-XF) or in serum-containing medium (Control).
- Culture plates were coated with MesenCult®-XF Attachment substrate (STEMCELL Cat. #05425), which is essential for culturing MPCs in MesenCult®-XF medium (STEMCELL Catalog #05420).
- Clonogenic growth of MPCs was analyzed by plating primary BM-derived mononuclear cells at low densities in a 6-well plate (1.5 to 5.0 x 10^5 cells/well in MesenCult®-XF or at 2.5 x 10^5 to 1 x 10^6 cells/well in Control media). MPCs were stained with Giemsa stain and CFU-F derived colonies were enumerated.
- Expansion assays were performed by plating primary BM mononuclear cells in MesenCult®-XF at 3.0 - 7.0 x 10^4 cells/cm^2 and at 1 - 4 x 10^5 cells/cm^2 in Control medium. At each passage MPCs were dissociated with MesenCult®-Dissociation kit (STEMCELL Cat. #05426), which is essential for subculture of cells in MesenCult®-XF. MPCs were then re-platted at 1.5 - 4 x 10^5 cells/cm^2 for cultures in MesenCult®-XF and at 0.5 - 1 x 10^6 cells/cm^2 for cultures in Control medium.
- Chondrocyte differentiation was examined by transferring 5 x 10^5 cells previously expanded in either MesenCult®-XF or in Control medium in micromass culture to MesenCult®-chondrocyte differentiation medium and culturing the cells for 3-4 weeks. Alcian Blue and Collagen II staining were used to confirm chondrogenic differentiation.
- Cell surface phenotype of culture expanded cells in both MesenCult®-XF and Control was analyzed by FACS at P1 and P3 to assess MPCs purity.
- Immunosuppressive effects of MPCs generated in MesenCult®-XF or in Control medium were tested in a co-culture assay. MPCs were co-cultured with T-cells purified from peripheral blood and fluorescently labeled using carboxy-fluorescein-diacetate (CFSE). CFSE labeled T-cells (2 x 10^5 cells/well) were cultured with 1 x 10^5 or 1.25 x 10^5 MPCs. T-cells were activated by the addition of antibodies to CD3ε, CD28, and CD2. On days 3 and 7, cells were harvested and the T-cell division history analyzed by flow cytometry.

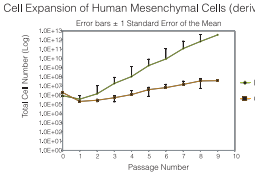
Results

TABLE 1: CFU-F-Derived Colony Frequency and Size (Average From All Seeding Densities) of MPCs Cultured in MesenCult®-XF and Control Medium

CFU-F/10^6 BM MNCs Mean ± SD; n = 6		CFU-F Size (mm) Average diameter ± SD; n = 3 (Range)	
Control	MesenCult®-XF	Control	MesenCult®-XF
76 ± 44	88 ± 54	2.8 ± 0.99 (1.5 - 6)	5.7 ± 0.3 (2.3 - 11)

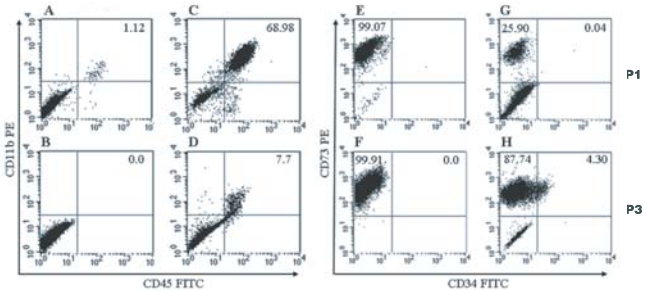
The frequencies of CFU-F derived colonies in both media was comparable. However, colonies generated in MesenCult®-XF were on average twice as large as those cultured in serum-containing medium.

FIGURE 1: Expansion of Human BM-Derived MPCs Cultured in MesenCult®-XF and FBS Containing Medium (Control)



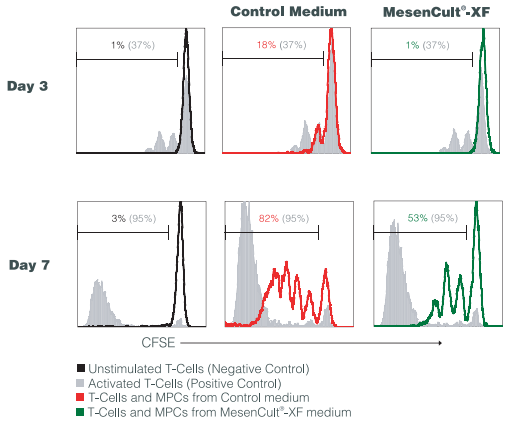
MPCs were cultured for 9 passages in MesenCult®-XF with an average cell expansion of 8.5 ± 1.4 fold; mean ± SD (n=3), at each subculture. In comparison, cells cultured in Control medium exhibited a comparatively lower proliferation rate. The average expansion of cells in Control medium was 2.7 ± 0.8 fold; mean ± SD (n=3), at each subculture. Several commercially available serum-containing media gave similar results as the Control medium.

FIGURE 2: MPCs Cultured in MesenCult®-XF Contain Less Hematopoietic Cell Contamination Compared to MPCs Cultured in Control Medium



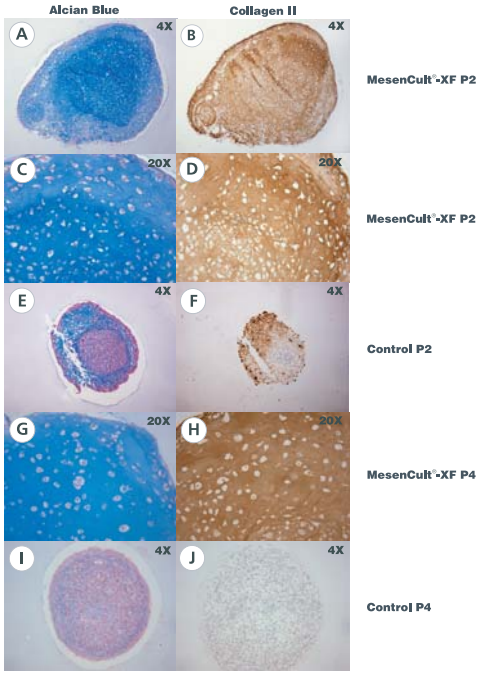
MPCs cultured in MesenCult®-XF and stained at first passage (P1,A) exhibit significantly less hematopoietic cell contamination than cells cultured in control medium for 1 or 3 passages (C and D, respectively). Similarly, staining of CD73 shows that at P1 (E) and P3 (F) MesenCult®-XF cultured cells consist of >90% of MPCs cultures, as compared to 26% and 88% of cells in control cultures at P1 (G) and P3 (H), respectively.

FIGURE 3: MPCs Cultured in MesenCult®-XF Suppress T-Cell Proliferation and Reduce Cell Cycle Division More Robustly Than MPCs Cultured in Serum-Based Medium



Passage 2 MPCs generated in MesenCult®-XF or a traditional serum-based medium were treated with mitomycin C prior to co-culture with T-cells. T-cells were purified from peripheral blood using EasySep® (STEMCELL Catalog #19051) immunomagnetic separation and fluorescently labeled using 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (5(6)CFDA,SE; CFSE). 2 x 10^5 CFSE-labeled T-cells were cultured with 1 x 10^5 MPCs 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 anti-CD45 antibody and propidium iodide and the T-cell division history measured as CFSE dye dilution analyzed by flow cytometry.

FIGURE 4: Enhanced Chondrogenic Differentiation of Human BM-Derived MPCs Expanded in MesenCult®-XF



Differentiation of cultured MPCs into the chondrogenic lineage was examined via micromass culture. Cells cultured in MesenCult®-XF for 2 passages (A,B,C,D) showed strong chondrogenic differentiation compared to cells cultured in serum-containing Control media for 2 passages (E,F). At P4, MesenCult®-XF MPCs maintained robust chondrogenic potential (G,H) whereas Control MPCs had lost chondrogenic potential completely (I,J).

Conclusions

- MPCs cultured in MesenCult®-XF showed greater expansion with reduced hematopoietic cell contamination at early passage compared to MPCs in Control medium.
- MPCs cultured in MesenCult®-XF showed robust immunosuppressive activity.
- MPCs previously cultured in MesenCult®-XF exhibited enhanced chondrogenic differentiation potential compared to MPCs in Control medium.

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