

Expansion of mesenchymal progenitor cells from human bone marrow in a novel serum and animal-free culture medium

Ravenska Wagey¹, Brenton Short^{1,2}, Betty Hoac¹, Terry Thomas¹, Allen Eaves^{1,3} and Bert Wognum¹.

¹STEMCELL Technologies Inc., Vancouver, BC, Canada, ²Dept. of Cellular Physiology, University of British Columbia Vancouver, BC, Canada, ³Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada.

Introduction

Human mesenchymal progenitor cells (MPCs) are a phenotypically and functionally heterogeneous cell population typically isolated from bone marrow aspirates. Given the innate ability of these cells to give rise to multiple tissue types including bone, fat and cartilage, as well as their efficacy in the modulation of immune disorders such as graft versus host disease (GVHD), there is considerable interest in utilizing MPCs in a broad repertoire of cell-based therapies for the treatment of human disease.

MPCs are typically cultured in medium containing fetal bovine serum which is a cause for concern when the cells are to be used in clinical applications, as potential incorporation of bovine derived proteins may lead to immunogenicity and rejection in transplant recipients. Traditional serum-containing medium is also problematic in that it facilitates not only the expansion of MPCs but also of contaminating plastic adherent hematopoietic cell populations which may persist for several passages.

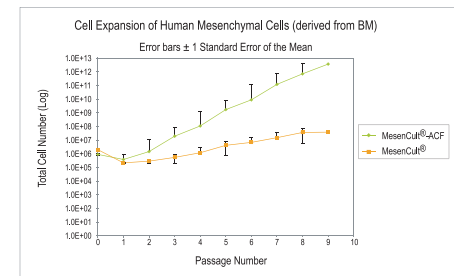
We have developed a humanized, serum and animal-component free medium (MesenCult®-ACF) formulation which promotes superior clonogenic growth and supports long-term expansion whilst maintaining multi-lineage differentiation potential of human bone-marrow derived mesenchymal cells in culture. Importantly, MPCs cultured in MesenCult®-ACF are essentially devoid of all hematopoietic contaminants at first passage.

Materials and Methods

- Culture plates were coated with MesenCult®-ACF Attachment substrate (Cat.#05425) which is essential for culturing MPCs in MesenCult®-ACF medium (Cat.#05420).
- Clonogenic growth of MPCs was analyzed by plating cells at low densities in 6-well plates. BM-derived mononuclear cells were plated at concentrations of $1.5 - 5.0 \times 10^5$ cells/well in MesenCult®-ACF or at 2.5×10^5 to 1×10^6 cells/well in serum-containing MesenCult®. Culture expanded MPCs were plated at concentrations of 25-200 cells/well in MesenCult®-ACF or at 100 to 1250 cells/well in MesenCult®. After 8-14 days of culture, MPCs were stained with Giemsa stain and CFU-F derived colonies were enumerated.
- Expansion assay was performed by plating Primary BM mononuclear cells in MesenCult®-ACF between $3.0-7.0 \times 10^4$ cells/cm² and $1.5 - 4 \times 10^3$ cells/cm² for passaged cells. Primary BM mononuclear cells in MesenCult® were plated at $1 \times 10^5 - 4 \times 10^5$ cells/cm² and $5 \times 10^3 - 1 \times 10^4$ for passaged cells. At each passage MPCs were dissociated with MesenCult®-Dissociation kit (Cat.# 05426) which is essential for subculture of cells in MesenCult®-ACF.
- Differentiation assays of MPCs into adipocytes, osteogenic cells and chondrocytes were performed with cells cultured at various passage number. Oil Red O, Alizarin Red and Alcian Blue stains were used to confirm the presence of adipocytes, osteogenic cells and chondrocytes.
- Cell surface phenotype of culture expanded cells in both MesenCult®-ACF and MesenCult® was analyzed by FACS at P0, P2, P4, P6 and P8.

Results

Fig.1. Expansion of Human BM-derived MPCs cultured in MesenCult®-ACF and FBS containing MesenCult® media



MPCs were cultured for 9 passages in MesenCult®-ACF with an average cell expansion of 8.5 ± 1.4 fold; mean \pm SD (n=3), at each subculture. In comparison, cells cultured in a range of commercially available serum-containing media or in MesenCult® exhibited a comparatively lower proliferation rate. The average expansion of cells in MesenCult® was 2.7 ± 0.8 fold; mean \pm SD (n=3), at each subculture.

Table 1. CFU-F-derived colony frequency and size (average from all seeding densities) of MPCs cultured in MesenCult®-ACF and MesenCult® media

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

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

Fig. 2. Representative of CFU-F colonies cultured in MesenCult®-ACF (A) and MesenCult® media (B)

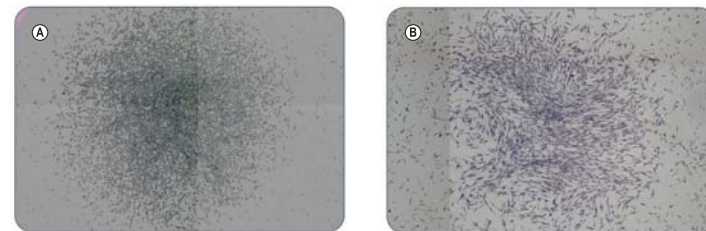


Table 2. CFU-F frequency of passaged cells cultured in MesenCult®-ACF and MesenCult® media

	MesenCult®-ACF Passage #				MesenCult® Passage #		
	Cells plated/well (6-well plate)	Total Counted CFU-F	Frequency (CFU-F/Total Cells)		Cells plated/well (6-well plate)	Total Counted CFU-F	Frequency (CFU-F/Total Cells)
P1	25	4.0	1/7	P1	100	1.0	1/34
	50	9.0			250	7.0	
	75	11			500	18	
	100	13					
P3	50	8.0	1/9	P3	100	7.0	1/18
	100	11.0			250	22.0	
	150	13			500	37	
P5	25	2.0	1/10	P5	100	6	1/20
	50	6.0			250	14	
	100	10			500	22	

More than 10% of MPCs at P1, P3 and P5 cultured in MesenCult®-ACF were able to form colonies. In comparison only 3-6% of MPCs cultured in MesenCult® formed colonies.

Fig.3. BM-derived MPCs cultured in MesenCult®-ACF (A) contain less hematopoietic contamination at early culture phase (P0) compared to MPCs cultured in MesenCult® medium (B).

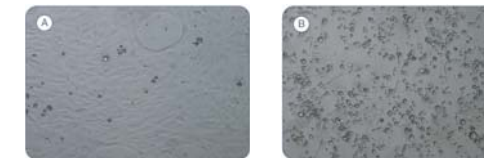
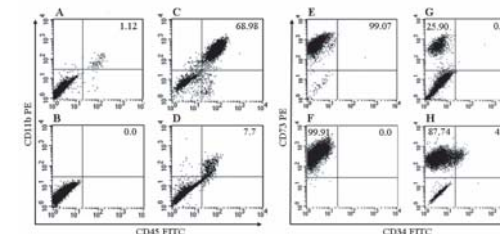


Fig.4. MPCs cultured in MesenCult®-ACF (A, B) contain less hematopoietic contamination compared to MPCs cultured in MesenCult® medium (C, D) at P0 and P2 culture phase.



Cells cultured in MesenCult®-ACF contain 1% and 0% double positive cells (CD45 and CD11b) at P0 and P2, respectively. Cells cultured in MesenCult® contain 69% and 8% double positive cells (CD45 and CD11b) at P0 and P2, respectively. Staining of cells in MesenCult®-ACF (E, F) and MesenCult® (G, H) for CD73 gave 99% (P0) and 99.9% (P2) positive cells and 26% (P0) and 88% (P2) positive cells, respectively.

Fig.5. Differentiation of Human BM-derived MPCs expanded in MesenCult®-ACF

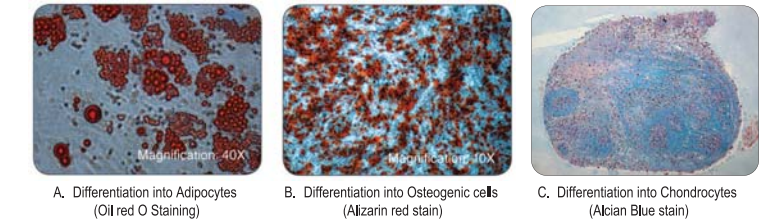
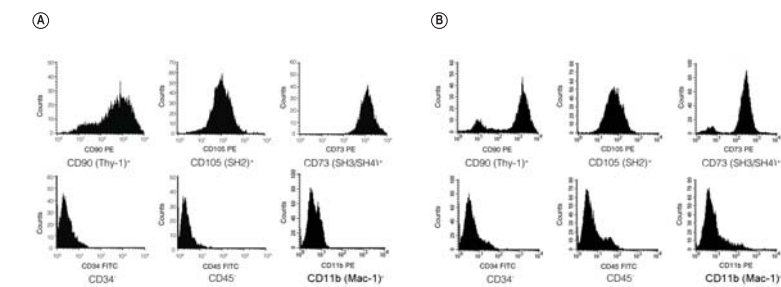


Fig. 6. FACS analysis of BM-derived MPCs (P2) in MesenCult®-ACF (A) and MesenCult® (B) media.



Conclusions

- MesenCult®-ACF is a defined serum-free formulation with no animal-derived components which promotes superior clonogenic growth and expansion of BM-derived and cultured MPCs.
- MPCs cultured in MesenCult®-ACF showed greater expansion (>8 passages) compared to serum-based MesenCult® while maintaining multi-lineage differentiation capacity.
- MPCs cultured in MesenCult®-ACF have less hematopoietic contamination at early passage compared to serum-based MesenCult®.
- MPCs cultured in MesenCult®-ACF have a surface marker phenotype characteristics of MPCs: CD90⁺, CD105⁺, CD73⁺, CD34⁻ and CD45⁻



WWW.STEMCELL.COM
 IN NORTH AMERICA TOLL-FREE TEL: 1 800 667 0322 • TOLL-FREE FAX: 1 800 567 2899
 TEL: 1 604 877 0713 • FAX: 1 604 877 0704 • EMAIL: INFO@STEMCELL.COM
 TOLL-FREE TEL: 00 800 7836 2355 • TOLL-FREE FAX: 00 800 7836 2300
 IN EUROPE TEL: +33 (0) 4 76 04 75 30 • FAX: +33 (0) 4 76 18 99 63 • EMAIL: INFO.EU@STEMCELL.COM
 IN AUSTRALIA TOLL-FREE TEL: 1 800 060 310 • TEL: +61 (0) 7 5474 5042 • FAX: +61 (0) 9338 4320 • EMAIL: INFO.AUS@STEMCELL.COM
 IN SINGAPORE TEL: (65) 972 6660 • EMAIL: INFO.SG@STEMCELL.COM