A novel animal component-free culture medium optimized for derivation and expansion of mesenchymal cells from bone marrow and adipose tissues

Ravenska Wagey[1], Jacky Yau[1], Melissa Elliot[1], Matthew Wong[1], Erik Hadley[1], Chris Duronio[1], Arthur Sampaio[1], Bari Zahedi[1], Cindy Miller[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]

[1] STEMCELL Technologies Inc., Vancouver, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, B.C., Canada

Introduction

Human mesenchymal progenitor cells (MPCs) derived from bone marrow aspirates or adipose tissues are routinely expanded *ex vivo* in culture systems containing bovine or human serum. In recent years, serum-free cell culture media containing exclusively human-derived materials (termed xeno-free) have been developed for use in both research and clinical applications. It is anticipated that the next advancement towards using fully-defined, animal component-free (ACF) medium formulations and culture matrices for *ex vivo* expansion of MPCs will help address the safety concerns associated with exposure to animal-derived materials and minimize the potential risks of secondary immune rejections[1]. Here we describe MesenCult™-ACF, the first fully-defined, serum- and animal component-free culture medium and compatible defined matrix, which supports efficient derivation and expansion of MPCs from primary human bone marrow (BM) and adipose tissues (AD). We compared the performance of the new MesenCult™-ACF (referred as M-ACF) medium to standard serum-based medium as the control (referred as SC) in MPCs derived from primary bone marrow and adipose tissues. Our data shows that the complete MesenCult™-ACF system is highly efficient at supporting cell attachment, clonogenic growth and long-term expansion of MPCs directly from primary bone marrow and adipose tissues under strictly animal component-free culture conditions without any serum requirement.

Materials and Methods

Matrix-Coated Plasticware

Plasticware for culturing MPCs in MesenCult™-ACF medium were pre-coated with MesenCult™-ACF two hours prior to usage.

Cells and Media

Primary human BM was processed by Ficoll® density separation and isolation procedure performed without the use of animal-derived reagents. BM-mononuclear cells were set up in colony-forming unit fibroblast (CFU-F) assays and expansion cultures in MesenCult™-ACF (M-ACF), serum control (SC) medium, Medium A, or Medium B. Medium A and Medium B are commonly used commercially-available defined media. AD tissues from human mammoplasty reduction were digested with collagenase type I to obtain a single-cell suspension of the stromal vascular fraction (SVF) and were set up for CFU-F and expansion assays in M-ACF, SC, or in Medium A.

Clonogenic Growth

Clonogenic growth of AD-derived MPCs was evaluated in CFU-F assays by plating SVF derived cells in multiple wells of 6-well plate at low seeding densities (ranging from 500 - 10,000 cells/cm^2) in M-ACF, SC medium, Medium A, or Medium B. The CFU-F colony size and the size of individual cells within a colony were analyzed using the Image J software program[2].

Expansion Cultures

Expansion cultures were initiated by plating primary BM-mononuclear cells (MNCs) in M-ACF, SC medium, Medium A, or Medium B. BM-MNCs were plated at a range of 3 x 10^4 to 5.0 x 10^4 cells/cm^2 in M-ACF and at a range of 5 x 10^4 to 1.0 x 10^5 cells/cm^2 in SC medium in separate wells. Expansion cultures with primary AD-derived MPCs were plated at 500 - 1,250 cells/cm^2 in separate wells in either M-ACF or SC. For subsequent subculture, both BM and AD-derived MPCs were seeded at 1,500 - 3,000 cells/cm^2 and the proliferative potential of MPCs in each medium was determined by counting the total cumulative cell number obtained at each serial passage up to Passage 8 (P8) for both tissue sources.

In Vitro Differentiation Cultures

The multilineage potential of MPCs previously cultured in M-ACF medium was assessed in adipogenic, osteogenic and chondrogenic differentiation cultures. Adipogenic, osteogenic and chondrogenic cells were detected by Oil Red O, Von Kossa/Alkaline Phosphatase and Alcian Blue staining, respectively.

Phenotype Analysis

Cell surface phenotype of BM MNCs cultured in M-ACF was analyzed by flow cytometry for mesenchymal and hematopoietic markers.

Results

Bone Marrow-derived MPCs

TABLE 1: CFU-F-derived colony frequency of MPCs cultured in M-ACF, SC medium, Medium A, or Medium B

A)

CFU-F/10^6 BM MNCs	
MesenCult™-ACF	Serum Control
51 ± 8	29 ± 5

Mean ± SEM; n = 6

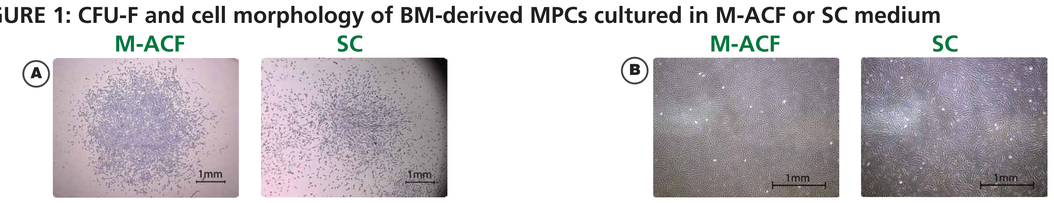
B)

CFU-F/10^6 BM MNCs		
MesenCult™-ACF	Medium A	Medium B
45 ± 2	10 ± 5	No CFU-F

Mean ± SEM; n = 3

CFU-F numbers were enumerated after 13 days from primary BM-mononuclear cells. **A)** Frequencies of CFU-F-derived colonies were significantly higher (two-tail, paired t-test; p<0.05) in M-ACF compared to SC medium. **B)** Frequencies of CFU-F-derived colonies were significantly higher (two-tail, paired t-test; p<0.05) in M-ACF compared to Medium A. Medium A and Medium B are commonly used commercially-available defined media.

FIGURE 1: CFU-F and cell morphology of BM-derived MPCs cultured in M-ACF or SC medium

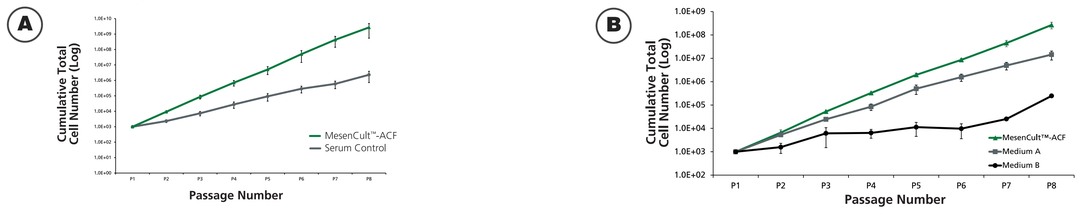


A) Representative images of CFU-F in M-ACF or SC medium stained with Giemsa. **B)** MPC morphology in expansion cultures (P2) containing M-ACF or SC medium. MPCs cultured in M-ACF medium are relatively smaller in size than MPCs cultured in SC media.

FIGURE 2: Size of BM-derived MPCs cultured in M-ACF or SC-media

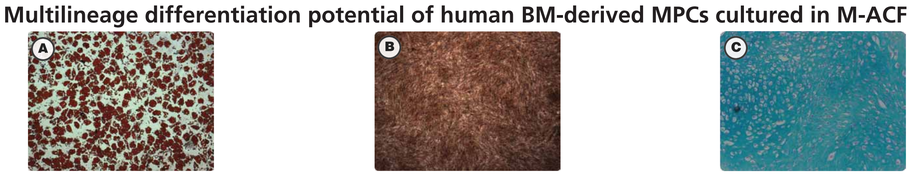
The average colony size was analyzed by number of cells within a colony. **A)** CFU-F-derived colonies in M-ACF had higher number of cells (3,098 ± 198 cells; mean ± SEM; n = 6) compared to SC (872 ± 144 cells; mean ± SEM; n = 6). **B)** The average size of individual cells within a colony when MPCs were cultured in M-ACF medium was smaller (74 ± 5 units; mean ± SEM; n = 6), compared to serum control (268 ± 28 units; mean ± SEM; n = 6). The small cell size in M-ACF medium is maintained at late passages and may indicate the maintenance of their "stemness" based on proliferation and differentiation properties[3,4]. *Two-tail, paired t-test; p<0.05.

FIGURE 3: Expansion of human BM-derived MPCs cultured in M-ACF, SC medium, Medium A, or Medium B



A) Average cell expansion of cultured BM-MPCs from P1 to P8 (31 to 46 days) in M-ACF medium was higher (6.9 ± 0.5 fold; mean ± SEM; n = 8), compared to serum control (2.7 ± 0.2 fold; mean ± SEM; n = 8) at each subculture. **B)** Average cell expansion of cultured BM-MPCs from P1 to P8 was higher in M-ACF (6.0 ± 0.5 fold; mean ± SEM; n = 3) compared to Medium A (4.0 ± 0.5; mean ± SEM; n = 3) or Medium B (2.5 ± 0.7; mean ± SEM, n = 3).

FIGURE 4: Multilineage differentiation potential of human BM-derived MPCs cultured in M-ACF medium



MPCs maintained in M-ACF medium for multiple passages are capable of differentiating into **A)** adipocytes (Oil Red O staining), **B)** osteogenic cells (Von Kossa/ALP staining) and **C)** chondrocytes (Alcian Blue + Nuclear Fast Red staining).

FIGURE 5: BM-derived MPCs cultures in M-ACF contains reduced CD45/11b$^+$ cells compared to SC medium at P1

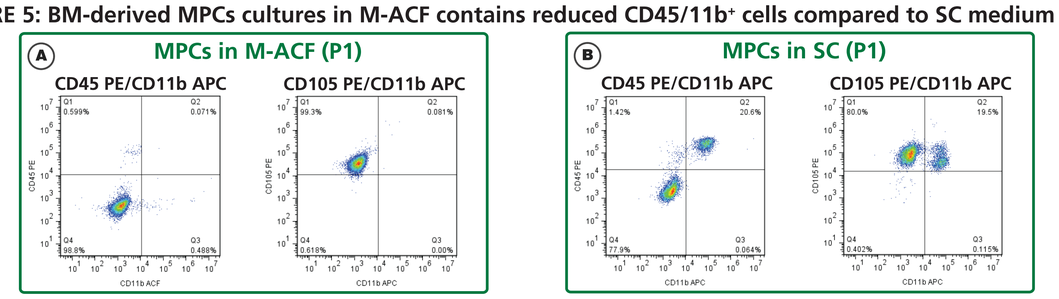
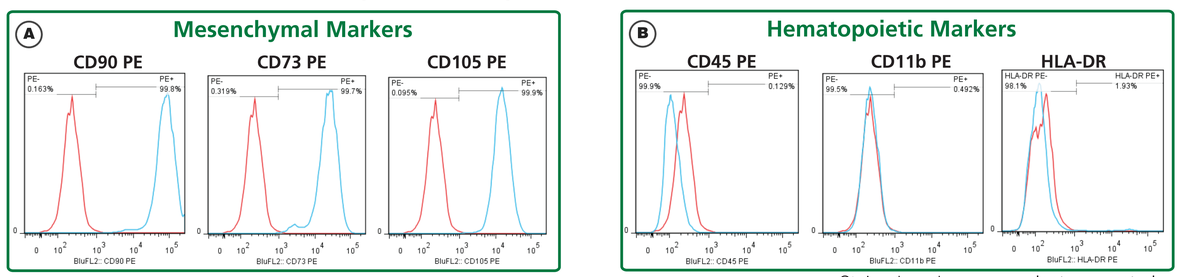


FIGURE 6: BM-derived MPCs cultured in M-ACF medium express high levels of mesenchymal markers (CD90, CD73, and CD105), but did not express hematopoietic markers (CD45, CD11b, and HLA-DR)



MPCs cultured in M-ACF were stained at Passage 4 with antibodies to **A)** mesenchymal markers (CD90, CD73, and CD105) and **B)** hematopoietic markers (CD45, CD11b, and HLA-DR).

Adipose-derived MPCs

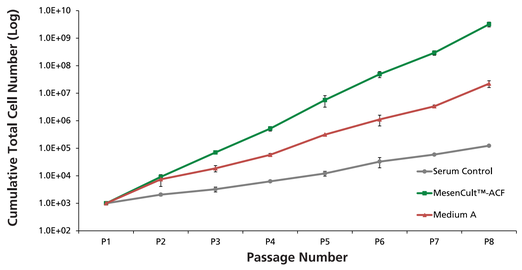
TABLE 2: CFU-F-derived colony frequency of AD-MPCs cultured in M-ACF, SC medium, or Medium A

CFU-F/10^6 SVF AD-MSC		
MesenCult™-ACF	Medium A	Serum Control
379 ± 60	281 ± 56	148 ± 32*

Mean ± SEM; n = 3 paired experiments

* Frequencies of CFU-F-derived colonies were significantly higher (two-tail, paired t-test; p<0.05) in M-ACF compared to the serum control. Medium A is a commonly used commercially-available defined medium.

FIGURE 7: Expansion of human AD-derived MPCs cultured in M-ACF, SC medium, or Medium A



Average cell expansion of cultured AD-MPCs from P1 to P8 (26 to 48 days) in M-ACF medium was higher (9.5 ± 0.9 fold; mean ± SEM; n = 3) compared to the serum control (2.0 ± 0.1; mean ± SEM; n = 3) or Medium A (4.4 ± 0.6; mean ± SEM; n = 3).

Summary

- MesenCult™-ACF is a novel medium formulation able to support highly efficient cell attachment, isolation and clonogenic growth directly from primary human BM and adipose tissue under fully-defined conditions.
- MPCs cultured in MesenCult™-ACF medium showed greater expansion than MPCs cultured in serum-containing medium while retaining multipotential differentiation capacity in long-term cultures.
- This is the first complete, fully-defined animal component-free culture system which produces high quality MPCs from primary bone marrow and adipose tissues without exposure to serum or xenogeneic components during *ex vivo* cell expansion, from cell processing to expansion.

[1] Horwitz EM, *et al.*, *Proc Natl Acad Sci U S A*, 2002 Jun 25; **99** (13):8932-7. [2] Abramoff MD, et al. Biophotonics International **11** (7): 36-42, 2004.
[3] Sekiya I, Larson BL, Smith JR, Pochampally R, Cui JG, Prockop DJ, *Stem Cells*, 2002; **20** (6):530-41. [4] Colter DC, Sekiya I, Prockop DJ, *Proc Natl Acad Sci U S A*, 2001 Jul 3; **98** (14):7841-7845.

STEMCELL™ TECHNOLOGIES