Development of Serum-Free Media for Mesenchymal Stem Cells, Complied with Japanese Standards for Biological Ingredients

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Introduction

Serum-containing culture media have been widely used for basic research and manufacturing of regenerative medicine products. However, serum has a potential risk of virus infection and adds lot-to-lot variability to cell culture process. To overcome these problems, serum-free media have been focused on cell culture process. In addition, in Japan, cell culture media for the production of regenerative medicine products are required to meet a Japanese regulation called “Standards for Biological Ingredients.” Its purpose is to ensure the quality, efficacy, and safety of products.

In this study, we have modified PRIME-XV MSC Expansion XSFM (Irvine Scientific Sales, Inc.) which is a xeno- and serum-free medium for expansion of human mesenchymal stem cells (hMSCs) to meet the Japanese regulation. The modified media were named as MSC Expansion XSFM B (XSFM B) and MSC Expansion XSFM C (XSFM C) and compared the cell growth, morphology, and immunophenotype with the original XSFM.

Materials and Methods

Cell Growth: hMSCs from ATCC and dental pulp stem cells (DPSCs) from Advanced Cell Technology and Engineering Ltd. were subcultured for 4 weeks. CellBIND® 6-well plates (Corning) were used for cell culture in XSFM B and C. Tissue culture plates precoated with and without fibronectin (Corning) were used in XSFM and serum-containing medium, respectively. Viable cells were counted by utilizing an automated cell counter (Vi-Cell® XR analyzer; Beckman Coulter Inc.). Population doubling level (PDL) and population doubling time (PDT) were calculated by the following equations.

$PDL = (\log N - \log N0) / \log 2$ $PDT = \log 2 \times \Delta t / (\log N - \log N0)$
N = Total number of cells harvested; N0 = Initial number of cells seeded.

Immunophenotype: Cell surface markers (CD45, CD90, CD105) were analyzed by flow cytometry (BD FACSVerse; BD Biosciences company).

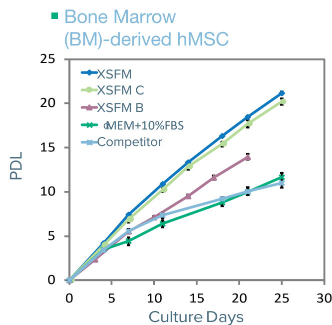
Differentiation: Cells were cultured for 3 weeks and stained using the following kits and reagents, respectively.
Adipogenic differentiation：hMSC differentiation BulletKit–Adipogenic (Lonza) and Oil Red O
Osteogenic differentiation：hMSC differentiation BulletKit–Osteogenic (Lonza) and Alizarin Red S
Chondrogenic differentiation: PRIME-XV Chondrogenic Differentiation XSFM (Irvine Scientific) and Alcian Blue

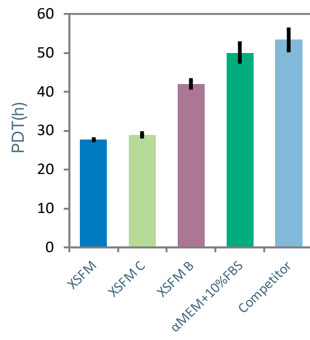
Karyotype analysis: Chromosomal G-band analysis of cells at passage 11 was performed at NIHON GENE RESEARCH LABORATORIES Inc.

Result 1 (hMSC)

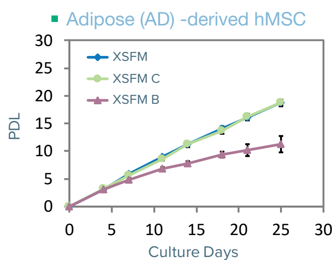
1. Cell Growth

▪ Bone Marrow (BM)-derived hMSC

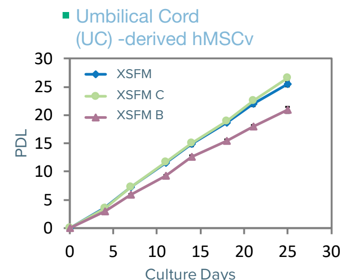




▪ Adipose (AD) -derived hMSC

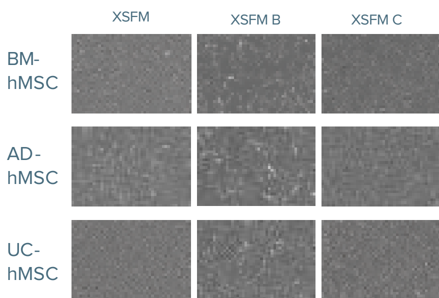


▪ Umbilical Cord (UC) -derived hMSCv



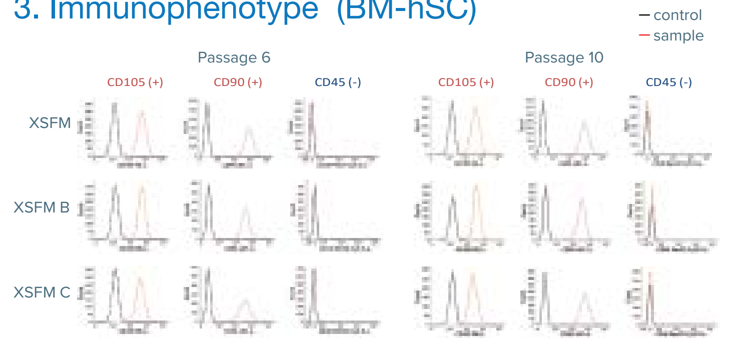
Similar cell growth was observed in both XSFM C and XSFM. But PDLs in XSFM B were 60–80% compared with those in XSFM depending on the cell source. XSFM B and C supported better cell growth than competitor's medium and serum-containing medium.

2. Morphology (Passage 10)



Similar cell morphologies were observed in XSFM C and XSFM, but XSFM B showed slightly different morphologies from XSFM.

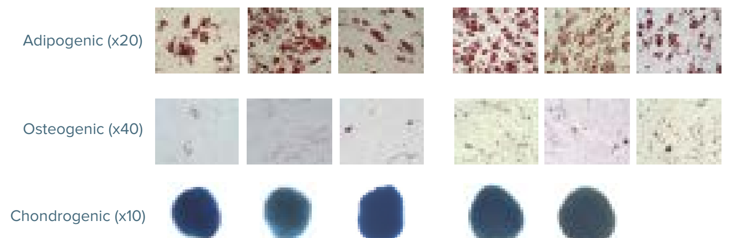
3. Immunophenotype (BM-hSC)



Same immunophenotypic profile for CD105, CD90, and CD45 was observed on hMSCs cultured in XSFM, XSFM B, and XSFM C.

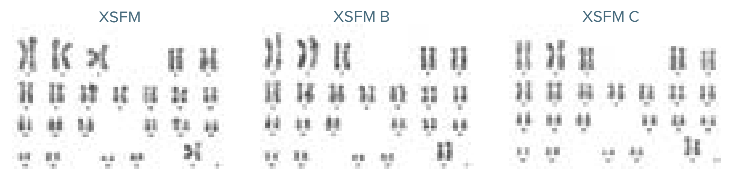
4. Differentation (BM-hSC)

▪ Adipogenic, Osteogenic: at passage 7 (Left), 11 (Right), Chondrogenic: at passage 6 (Left), 10 (Right)



BM-hMSCs in XSFM B and C were differentiated into adipocytes, osteoblasts and chondrocytes as is the case with XSFM.

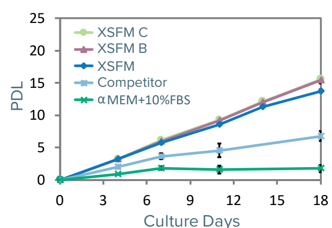
5. Karyotype Analysis (BM-hSC, Passage 11)

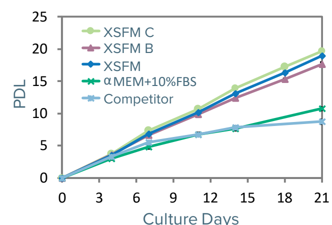


No gross chromosomal aberration was observed in hMSCs cultured using XSFM, XSFM B and C.

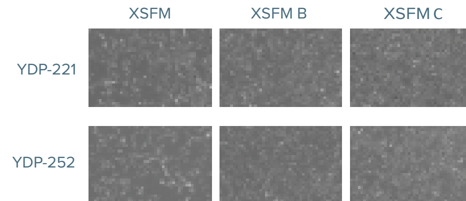
Result 2 (DPSC)

1. Cell Growth

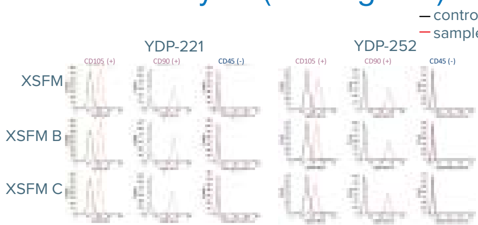




2. Morphology (Passage 13)



3. FACS Analysis (Passage 13)



The similar cell growth profiles were observed by the cell culture in XSFM B, C, and XSFM. No abnormality was observed in morphology and FACS profiles.

Conclusion

- To meet the Japanese regulation, XSFM was modified to XSFM B and C. (XSFM B and C were officially confirmed to meet the Japanese regulation by Pharmaceuticals and Medical Devices Agency).
- XSFM B and C were demonstrated to have equal performance compared to XSFM and better performance than serum-contained medium and competitor's medium.
- No negative impact on cell characteristics was observed by the media modification. However, some cell source dependence were observed by XSFM B on cell growth and morphology.
- This study indicates that XSFM B and XSFM C complied with the Japanese regulation could be widely applied for manufacturing of regenerative medicine products.

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