Defined Culture Conditions for Efficient Derivation, Expansion and Cryopreservation of Mesenchymal Progenitor Cells from Human Pluripotent Stem Cells

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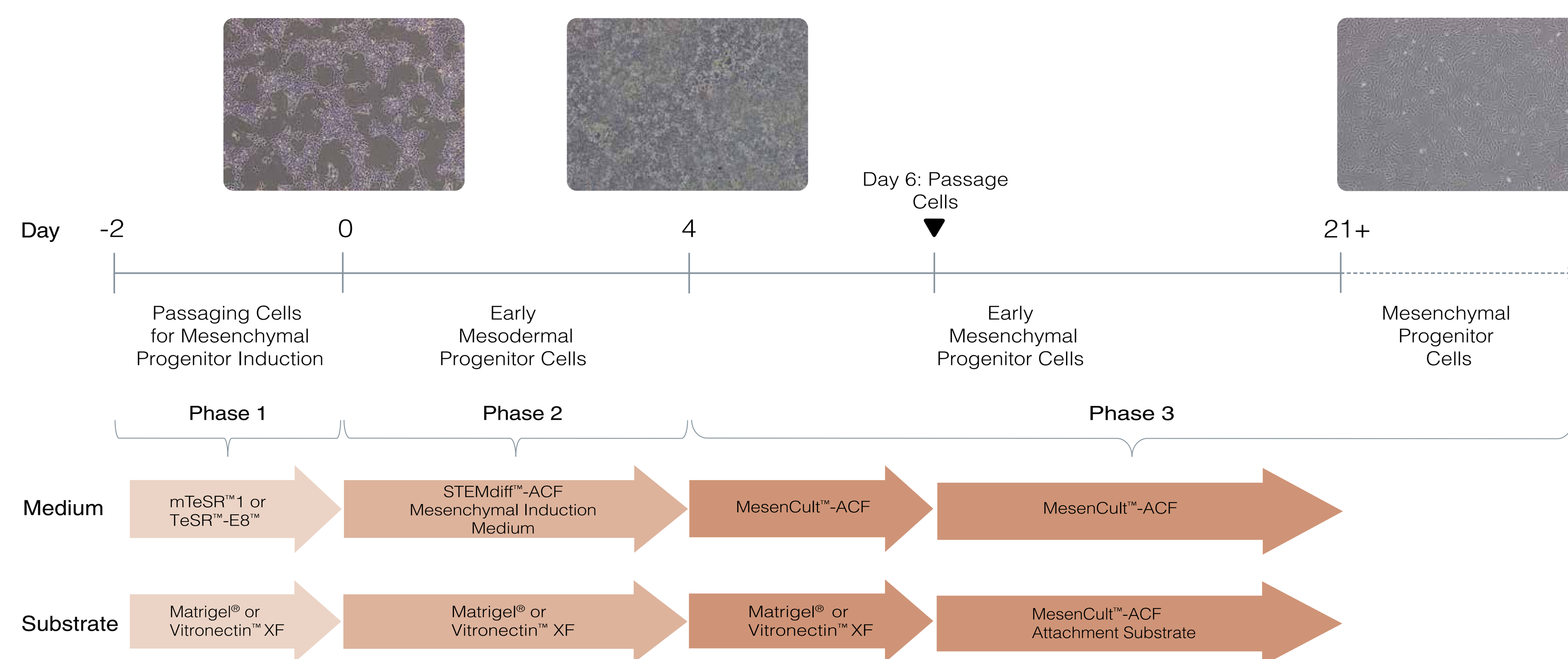
Introduction

Mesenchymal progenitor cells (MPCs) are typically isolated from adult bone marrow mononuclear cells. However, their low frequency, heterogeneity, and the invasive nature of isolation from adult tissues have prompted researchers to use human pluripotent stem cells (hPSCs) as an alternative source. Current approaches to derive MPCs from human induced pluripotent stem cells (hiPSCs) or human embryonic stem (hES) cells involve culturing cells in serum-containing medium on feeder layers of animal cells or using the embryoid body method but this requires extensive time in culture. We have developed a method to efficiently induce derivation of MPCs from hiPSCs and hES cells under defined serum-free and animal-component free (ACF) culture conditions.

Materials and Methods

Cells from hES (H9) and hiPSC lines (F016, F031) previously cultured in mTeSR™1 medium on Corning® Matrigel® or in TeSR™-E8 medium on Vitronectin™ XF were dissociated into single cells using Gentle Cell Dissociation Reagent (GCDR) and seeded at 5 x 10⁴ cells/cm² in monolayer cultures. These cells were differentiated into MPC-like cells using the protocol shown in **Figure 1**.

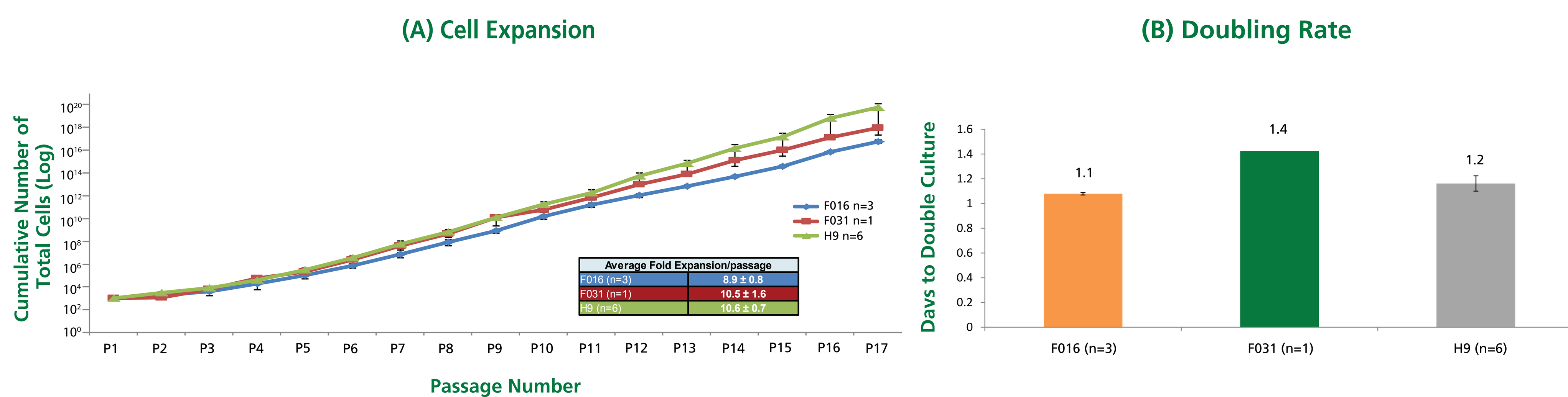
FIGURE 1: Derivation of MPCs from hiPSCs/hES cells in an animal-component free culture system



For initial passaging, cells were dissociated using Gentle Cell Dissociation Reagent (GCDR) (STEMCELL Technologies), counted and seeded at 1.5 - 10 x 10³ cells/cm²; subsequent passages, cells were seeded at 3 - 8 x 10³ cells/cm² on MesenCult™-ACF substrate. When cells started adopting MPC-like morphology (~P4), subsequent passages can be seeded at 1.5 - 3 x 10³ cells/cm². The proliferative potential of hPSC-derived MPCs in each medium was measured by counting cells at each passage for up to 17 passages. The growth curve was generated by multiplying the initial number of cells by fold expansion for each passage cumulatively. The doubling rate was calculated using the formula = N/(3.3*LOG(C_2/C_1)); N = number of days in culture; C_1 = initial cell concentration; C_2 = final cell concentration. Cell morphology and differentiation potential into adipocytes (Oil Red O staining), osteogenic (Alizarin Red staining) cells and chondrocytes (pellet assay; histology sections stained with Alcian Blue) were evaluated. Cell phenotype was analyzed by flow cytometry at different stages during the differentiation process. Cells cultured in MesenCult™-ACF medium were cryopreserved in MesenCult™-ACF Freezing medium at Passage 9 (hES-derived MPCs) and Passage 11 (hiPSC-derived MPCs). Post-thaw cell recovery and viability were calculated. Cell expansion of post-thaw cryopreserved cells was evaluated.

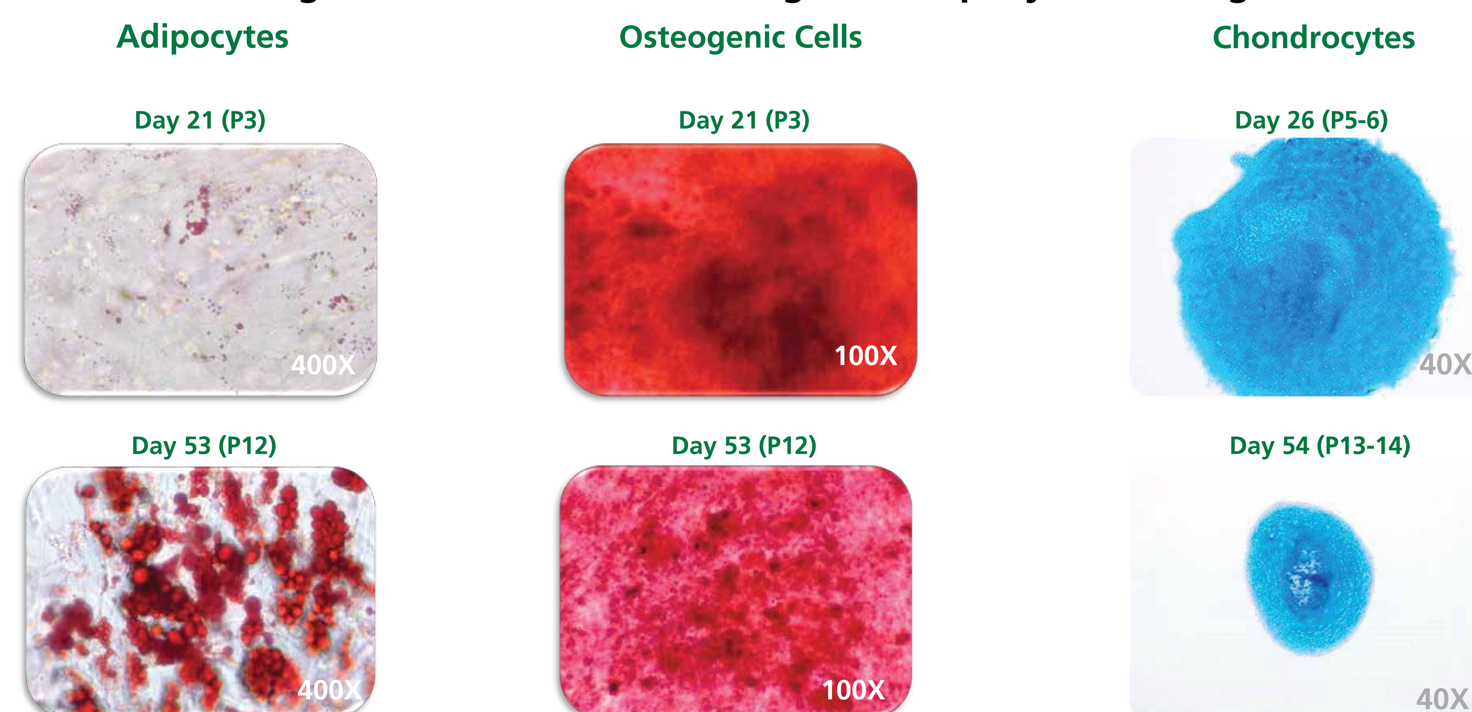
Results

FIGURE 2: (A) Cell expansion and (B) Doubling Rate of MPCs derived from hiPSCs (F016 and F031) and hES (H9) cells in MesenCult™-ACF culture medium



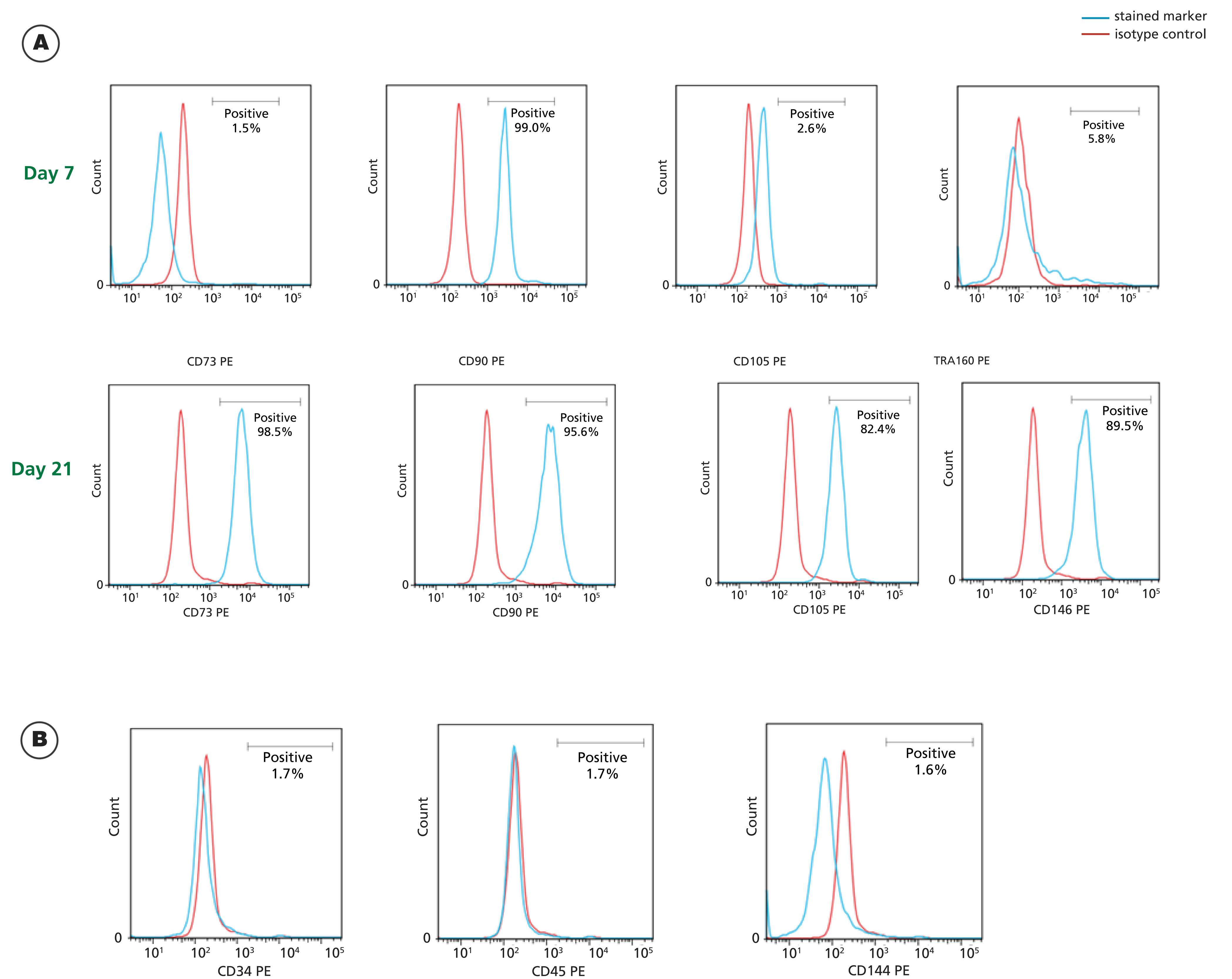
The average cell expansion per passage over 17 passages for MPCs derived from hES (H9) and hiPSCs (F016 and F031) was about 9 and 10 fold, respectively (A). The time to double cell number for hES and hiPSC-derived MPCs ranged from 1.1 - 1.4 days (B).

FIGURE 3: Representative images of MPCs differentiating into adipocytes, osteogenic cells, and chondrocytes



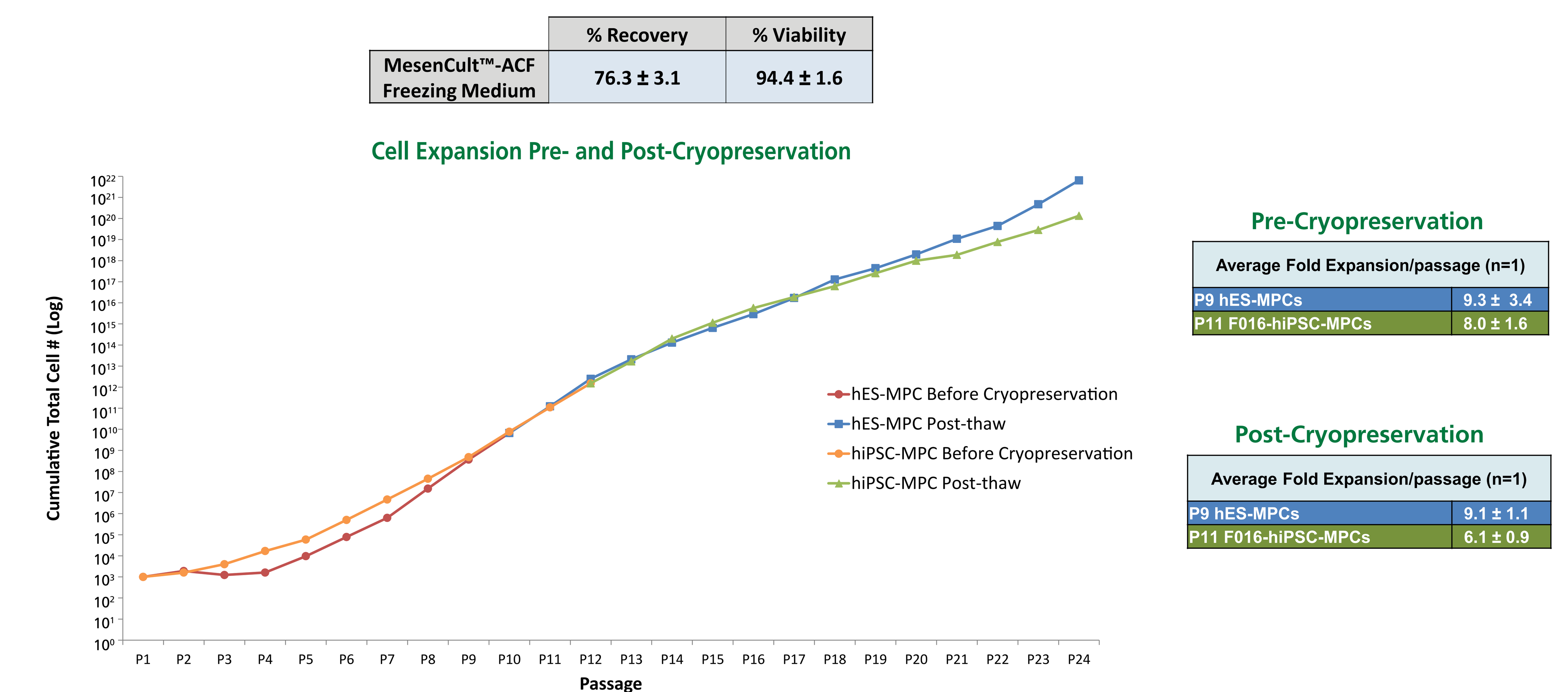
hiPSC-derived MPCs cultured in MesenCult™-ACF at early and late passsages were differentiated in the appropriate differentiation culture conditions into adipocytes, osteogenic cells and chondrocytes, as visualized by Oil Red O, Alizarin Red and Alcian Blue staining, respectively.

FIGURE 4: Representative flow cytometric analysis of MPCs on day 7 (before adopting mesenchymal-like cell morphology) and on day 21 (adopted MPC-like cell morphology)



Day 7 of hPSC-MPC derivation, the cells do not express markers associated with mesenchymal phenotype (CD73, CD105) and have lost the pluripotency marker Tra-160 (<10%). Prior to adopting MPC-like cell morphology, these cells expressed CD90. By day 21 hiPSCs-derived MPC-like cells cultured in MesenCult™-ACF medium expressed high levels of mesenchymal markers, (CD105, CD73, CD90) and the perivascular marker CD146 (A), but did not express hematopoietic (CD45, CD34) and endothelial (CD144) markers (B). hiPSC-derived MPC-like cells accumulate expression of MPC markers over time, except for CD90.

FIGURE 5: Cell expansion of hiPSC-derived MPC-cultured in MesenCult™-ACF and cryopreserved in MesenCult-ACF Freezing medium



MPCs derived from hPSC were culture expanded then cryopreserved in MesenCult™-ACF Freezing medium. These cryopreserved cells were then thawed and further expanded in MesenCult™-ACF medium for another 15 passages.

Summary

- We have developed a workflow to efficiently derive (in ~20 days) and expand MPCs from hiPSCs and hES cells in culture using defined media, substrate and dissociation solution
- hiPSC/hES cell-derived MPCs can be expanded long-term in MesenCult™-ACF medium
- hiPSC/hES cell-derived MPCs can differentiate into adipogenic, osteogenic and chondrogenic cells *in vitro*
- hiPSC/hES cell-derived MPCs (after 21 days in culture) express MPC (CD73, CD105 and CD90) and perivascular (CD146) markers and lack the expression of hematopoietic, endothelial and embryonic markers
- hiPSC/hES cell-derived MPCs can be cryopreserved in MesenCult™-ACF Freezing Medium with high viability upon thawing
- Studies are underway to characterize other hES and hiPSC lines

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