Suspension Culture Of Human Mesenchymal Stromal Cells On Dissolvable Microcarriers In An Animal Component-free Culture System

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INTRODUCTION

Mesenchymal stromal cells (MSCs) are currently used in many clinical trials with a required dose of ~ 1 - 4 million cells per kilogram patient mass, or ~ 100 million cells per patient per dose. Traditional monolayer culture can be labor intensive to meet this demand, therefore suspension culture has become a more efficient method for scale up of cells. Additionally, human and animal components in the culture system have the potential to introduce pathogenic components and increase lot-to-lot variability. Thus, scale up of MSCs in a defined animal component-free (ACF) workflow is a preferred culture system for clinical applications. MesenCult™-ACF Plus (MACF-P) is an ACF culture medium for the derivation and expansion of human MSCs optimized initially for monolayer culture. We have developed a complete ACF suspension culture system for scaling up bone marrow (BM)-derived MSCs using dissolvable microcarriers (DMC).

METHODS

Bone Marrow-Derived MSC Derivation, Expansion, Differentiation, and Phenotype

Monolayer culture
MSCs were isolated from human BM mononuclear cells (MNCs) using density gradient separation. Subsequently, a colony forming unit - fibroblast (CFU-F) assay was set up by plating BM MNCs at low seeding densities in MesenCult™-ACF Plus Medium. These cells were cultured on ACF Cell Attachment Substrate-coated culture flasks. MSCs were then subcultured until Passage (P) 1 or P2 and cryopreserved using MesenCult™-ACF Freezing Medium prior to setting up the MSC suspension culture.

Suspension Culture
Human BM-MSCs were seeded at 1500 cells/cm^2 with 2 g/L dissolvable microcarriers (DMCs) precoated by the manufacturer with Synthemax® II - a proprietary peptide acrylate polymer - in a 125 mL horizontal spinner flask. An intermittent agitation attachment protocol was used for the first 20 hours of culture (5 minutes at 25 rpm, 30 minutes rest) and then held at 30 rpm for the remainder of the culture. MSCs were cultured for 3 - 5 days until the cells reached ~70 - 80% confluency. MSCs were then dissociated from the DMCs by washing with phosphate-buffered saline (PBS), adding an optimized ACF harvest solution, and incubating at 37°C at 30 rpm for 10 minutes. The solution was quenched, and the resulting MSCs were centrifuged, counted, and reseeded.

Differentiation Assays
hBM-MSCs cultured by monolayer and suspension culture were differentiated into adipocytes and osteogenic cells using standard differentiation protocols, as visualized by Oil Red O and Alizarin Red, respectively.

Phenotype
The expression of CD105, CD73, CD90, and CD45 markers on hBM-MSCs cultured in monolayer and suspension were evaluated after 5 consecutive passages in the respective culture conditions.

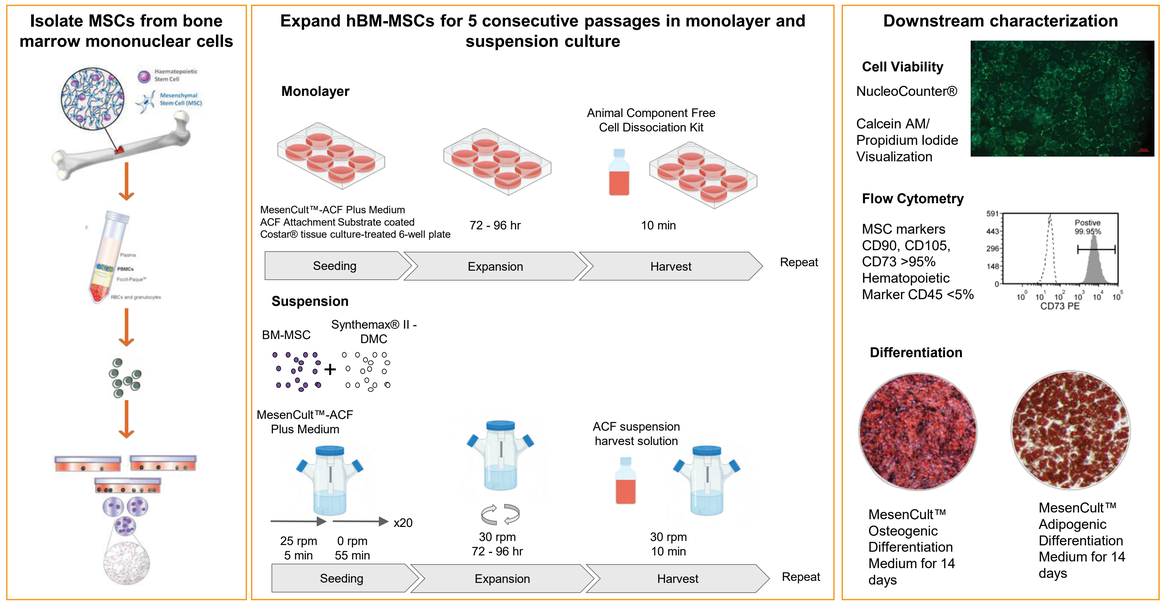
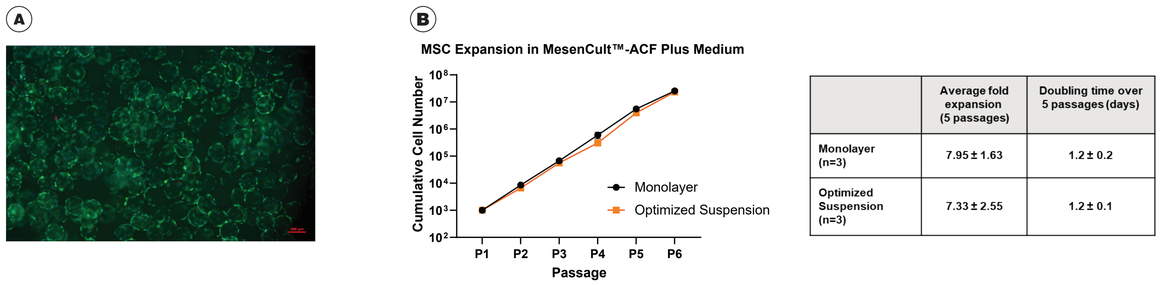


FIGURE 1. Human Bone Marrow-Derived MSCs: Isolation, In Vitro Expansion, and Characterization

RESULTS



	Average fold expansion (5 passages)	Doubling time over 5 passages (days)
Monolayer (n=3)	7.95 ± 1.63	1.2 ± 0.2
Optimized Suspension (n=3)	7.33 ± 2.55	1.2 ± 0.1

FIGURE 2. MesenCult™-ACF Plus Medium Supports Serial Passaging of hBM-MSCs in Suspension Culture

hBM-MSCs cultured in suspension on DMCs for 3 - 5 days in MesenCult™-ACF Plus Medium stained with Hoechst (blue), Calcein-AM (green), and Propidium Iodide (red). **(B)** hBM-MSCs expanded in suspension culture consistently and comparably to the monolayer control culture in MesenCult™-ACF Plus Medium.

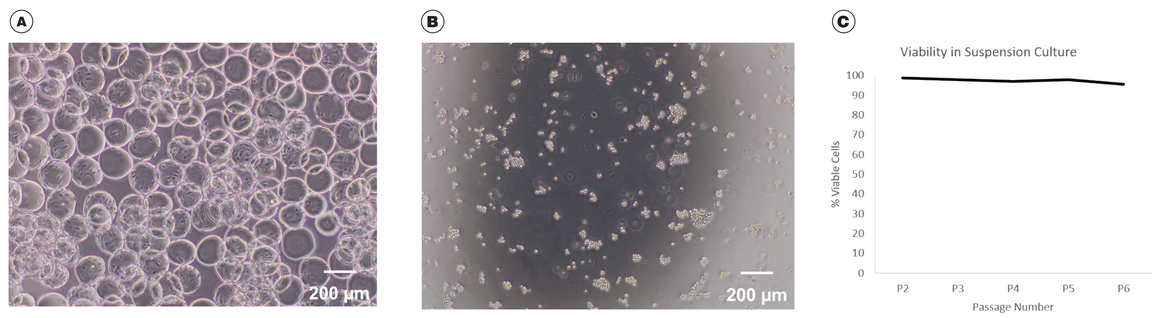


FIGURE 3. hBM-MSCs Can Be Detached Efficiently From Synthemax® II DMCs

(A) hBM-MSCs in MesenCult™-ACF Plus Medium were 70 - 80% confluent on the DMCs in suspension culture after 3 - 5 days. **(B)** Following 10 minutes of harvesting with the ACF harvest solution at 37°C, the DMCs were dissolved, and the hBM-MSCs were in a single-cell suspension. **(C)** The resulting cells maintained > 95% viability over 5 consecutive passages in suspension culture using this harvest method.

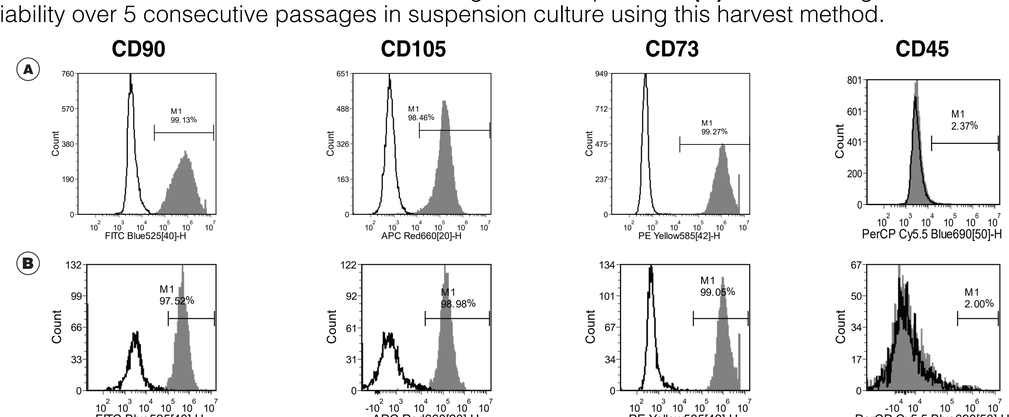


FIGURE 4. hBM-MSCs in MesenCult™-ACF Plus Medium Retain the MSC Phenotype After Serial Passaging in Both Monolayer and Suspension Culture

Following 5 consecutive passages in **(A)** monolayer or **(B)** suspension culture, the resulting hBM-MSCs maintained > 95% positive expression of the MSC phenotype markers CD90, CD105, and CD73 and remained negative (< 5%) for the hematopoietic marker CD45.

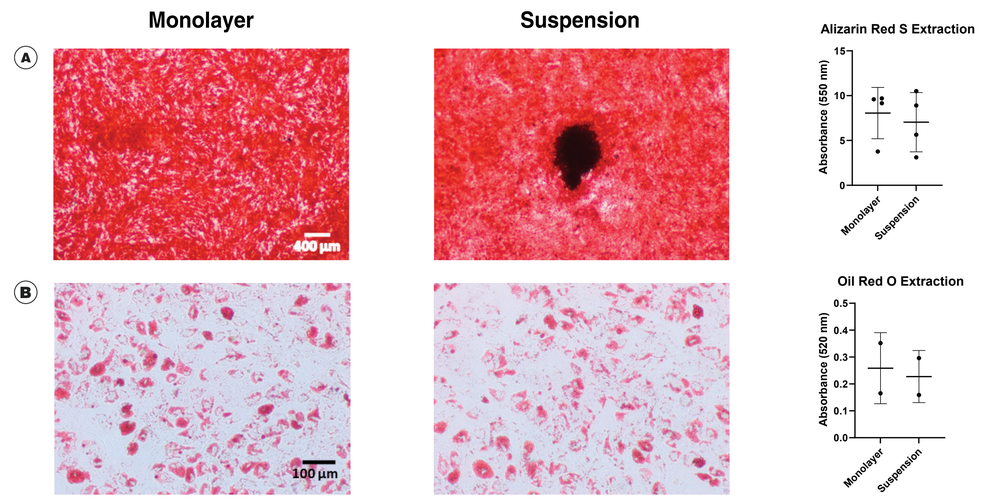


FIGURE 5. Adipocyte and Osteoblast Differentiation of hBM-MSCs After Serial passaging in Monolayer and Suspension Culture

After 5 consecutive passages in monolayer or suspension culture, hBM-MSCs had comparable osteogenic and adipogenic differentiation potential. **(A)** hBM-MSCs were differentiated using MesenCult™ Osteogenic Differentiation Kit (Human) following the standard 14-day differentiation protocol. The osteogenic differentiation was visualized by Alizarin Red S staining, extracted, and then quantified. **(B)** hBM-MSCs were differentiated using MesenCult™ Adipogenic Differentiation Kit (Human) following the standard 14-day differentiation protocol. The adipogenic differentiation was visualized by Oil Red O staining, extracted, and then quantified.

Summary

- hBM-MSCs in MesenCult™-ACF Plus Medium can be expanded in serial suspension culture using Synthemax® II DMCs with an average 1.2 ± 0.1 days doubling time for at least 5 passages
- hBM-MSCs can be harvested efficiently with high viability from Synthemax® II DMCs using an ACF suspension harvest solution
- hBM-MSCs retain their MSC phenotype, adipogenic and osteogenic differentiation potentials following serial suspension culture in MesenCult™-ACF Plus Medium on Synthemax® II DMCs comparably to the monolayer culture
- This data indicates robust expansion and efficient harvesting of hBM-MSCs in a complete ACF suspension workflow, which is a critical step in scaling up production of MSCs for cell therapy