A Microcarrier Cell Culture Process Using Novel Serum-Free Medium, Complying with the 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, the use of serum has several disadvantages including potential risk of virus infection and lot-to-lot variability. To overcome these problems, serum-free media have been developed by various media suppliers and many alternatives are now available on the market.

On the other hand, Pharmaceuticals and Medical Devices Agency (PMDA) of Japan requires all the raw materials used in Cell Therapy products to comply with its "Standards for Biological Ingredients". Therefore using serum-free media which conform thoroughly to the regulatory standards of PMDA is essential for the Cell Therapy drug developers.

We developed Prime-XV MSC XSFM MDF1 (MDF1), a novel serum-free medium complying with the PMDA regulations. MDF1 is formulated for a superior growth of hMSCs in monolayer cultures in comparison with both serum-containing medium and serum-free catalogue product of FUJIFILM Irvine Scientific. In this poster, we present the cell growth performance of MDF1 with hBM-MSCs using both monolayer culture and microcarrier culture technique. Furthermore, we present the performance of MDF3 (modified MDF1, serum-free, compliant with PMDA regulation), which was designed to improve the cell growth in microcarrier and the detachment efficiency.

Methods

Bone Marrow-Derived hMSCs from ATCC were used. 6 x 10^5 cells and 1.4 g cell-bind surface microcarrier (Corning, equivalent to the surface area of 500 cm^2) were suspended in 100 mL of both MDF1 and MDF3 medium and poured into 125 mL spinner flask (Corning). Following inoculation the culture was static for 1 hour, after which the culture was agitated constantly at 30 rpm at 37°C in humidified air containing 5% CO_2. 50% of the medium was exchanged at day 3 and day 5. After 7 days of culture, the cells were detached using with 50 mL of TrypLE Express (Thermo Fischer) for 10 mintues with 50-rpm agitation. After cell detachment, hMSCs were collected by vacuum filtration using 100 μm pluriStraner (pluriSelect Life Science). Viable cells were counted using with Vi-CELL XR analyzer (Beckman coulter). Surface marker analysis was performed by FACS Verse (BD Biosciences). All experiments were performed in triplicate.

Results

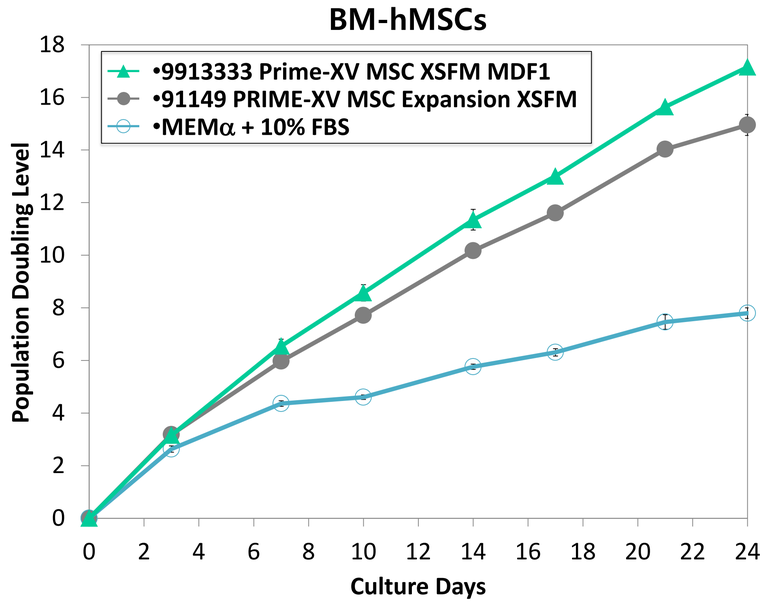

Figure 1. Growth kinetics of BM-hMSCs cultured in MDF1 in monolayer culture

A higher cell growth rate of BM-MSCs in monolayer culture was observed in MDF1 in comparison to XSFM and serum-containing medium.

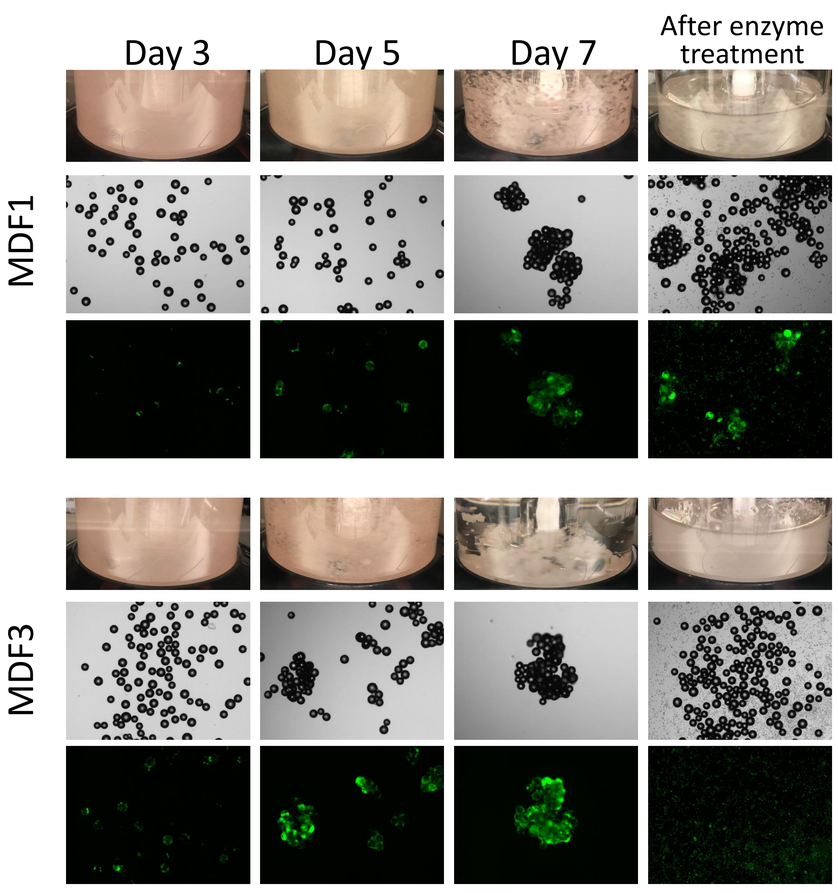

Figure 2. Appearance and image of microcarriers in spinner flask at day 3, 5, 7 and after enzyme treatment

Upper: Appearance of microcarriers in spinner flask
Middle: Bright-field microscopy image
Bottom: Fluorescent microscopy image of Calcein-AM stained cells

In the culture using with MDF3, the adhesion of cells on the surface of microcarriers was improved and cell-mediated bead aggregation was observed at an earlier time point.

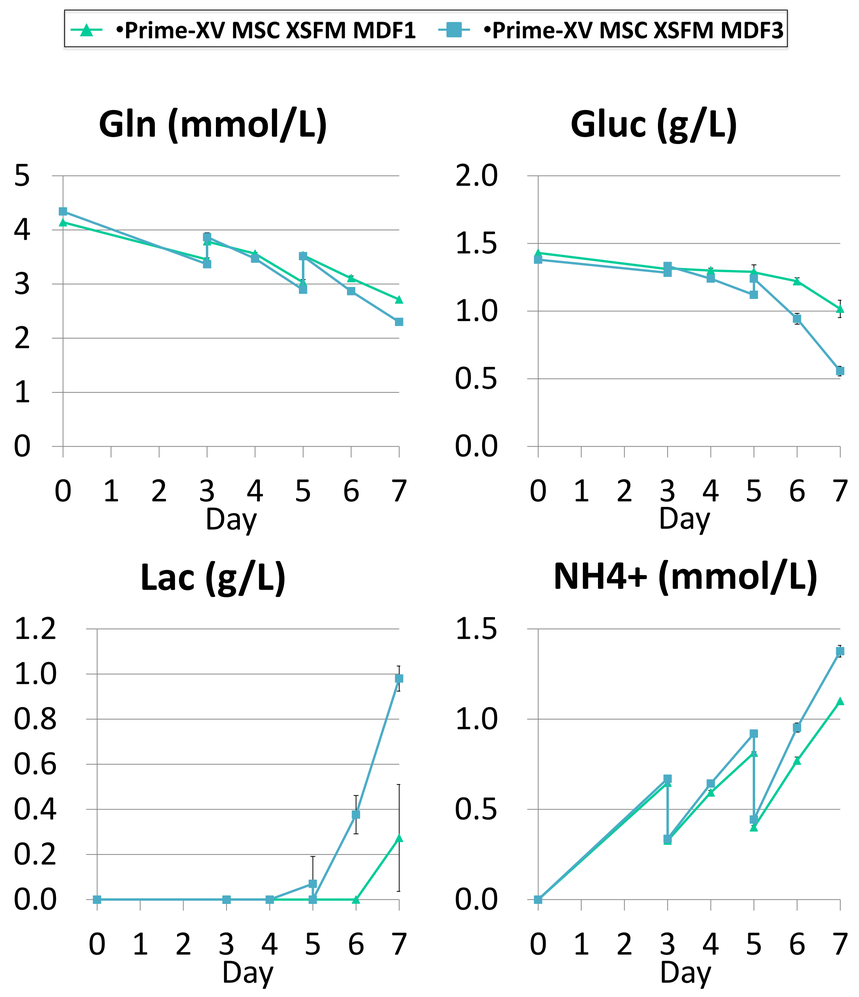

Figure 3. Nutrient consumption and metabolite production trends in the BM-hMSCs culture on microcarriers for 7-days of growth period

Higher glutamine and glucose consumptions as well as lactate and ammonium accumulations were observed in MDF3. This observation trends would likely be caused by the increased cell growth on the microcarriers with MDF3 medium.

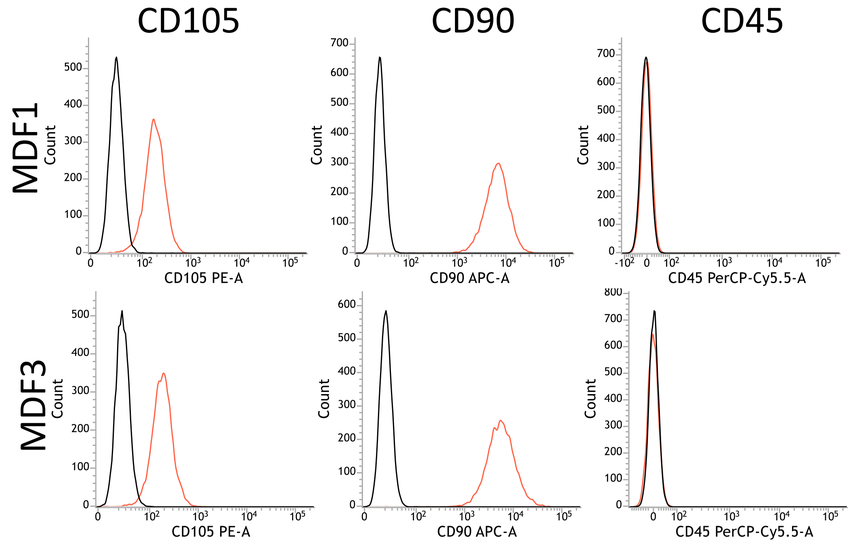

Figure 4. Cell surface marker expression of BM-hMSCs after 7-day culture on microcarriers

Immunophenotypic profiles of the BM-hMSCs cultured in MDF1 and MDF3 met the criteria of MSCs.

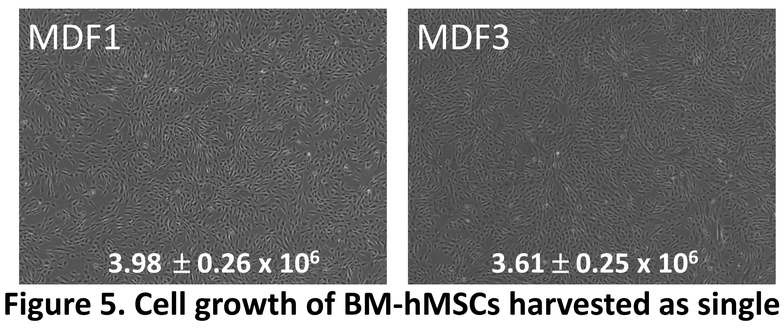

Figure 5. Cell growth of BM-hMSCs harvested as single cells after for 7 days of cultivation on microcarriers

Harvested hMSCs were seeded into 6-well plate at a density of 8 x 10^4 cells/well and cultivated for 3 days. Cell numbers are shown in the bottom of each picture. BM-hMSCs harvested from microcarriers grew normally and maintained the ability to differentiate into adipocytes, chondroblasts, and osteoblasts. (Data not shown)

Table Viable cell number, viability and PDL of BM-hMSCs after 7 days of cultivation on microcarriers

	Seeding viable cell density (1 x 10^5 cells / mL)	Viable cell density (1 x 10^5 cells / mL)	Viability (%)	Polulation doubling level (PDL)
MDF1	0.06	1.41 ± 1.4	96.5 ± 0.6	4.55
MDF3	0.06	3.34 ± 0.9	96.9 ± 0.4	5.79

BM-hMSC expanded more efficiently in MDF3 than in MDF1 and viable cell density in MDF3 reached to 3.34 x 10^5 cells/mL (PDL 5.79) after 7 days of cultivation on microcarriers.

Conclusions

- We developed Prime-XV MSC XSFM MDF1 (MDF1), a novel serum-free medium complying with the PMDA regulations. It was demonstrated that MDF1 achieved better cell expansion of BM-hMSCs than both serum-containing medium and XSFM of FUJIFILM Irvine Scientific in monolayer culture. Efficient cell expansion was also observed on cell-bind surface microcarrier without further pre-coating.
- It was further demonstrated that MDF3, modified of MDF1, showed better performance in expansion of BM-hMSCs on microcarrier. As a results of 7 days of cultivation on microcarriers, viable cell density was reached to 3.34 x10^5 cells/mL, which corresponds to PDL of 5.79.