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New And Efficient Method For Scaling Up Human Mesenchymal Stem Cells

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Abstract

The use of single layer T-flasks can prove to be time consuming, cumbersome and labor intensive for cell culture applications requiring large number of cells. Yet, caution needs to be exercised while scaling up cells using multi-layer vessels as differences in culture environment between layers of multi-layered vessels can affect yield, homogeneity and potential functional performance of cell populations. In the current study, we tested whether scaling up human mesenchymal stem cells (hMSCs) in BD Falcon™ Multi-Flasks affects their growth or characteristics compared to cells grown in control T-175 flasks.

BD Falcon Multi-Flasks have been designed with an equalization/mixing port that enables uniform distribution of cells and media on each layer of the culture vessel thereby providing a homogenous culture environment for cells throughout. hMSCs grown in media with and without serum, displayed no difference in morphology, attachment, proliferation, viability or recovery following culture in BD Falcon Multi-Flasks compared to cells cultured in control T-175 flasks. To verify impact of long-term culture, hMSCs were thawed directly in both vessel types and cumulative population doublings was determined for five consecutive passages. hMSC expansion potential was found to be equivalent per unit area in both vessel types. The ability of late passage cells to maintain multipotency and immunophenotype was also comparable between cells cultured in single and multi-layered vessels. In addition to the hMSCs, diverse established and primary cell lines have also been cultured in the BD Falcon Multi-Flasks with equivalent performance to T-175 flasks. This study demonstrates a new and efficient way to scale-up stem cells without the need for re-optimizing existing cell culture conditions or compromising the quality, performance and homogeneity of cell populations.

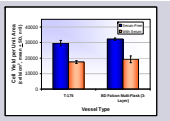
Materials and Methods

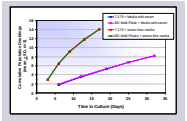
Poietics® Human Mesenchymal Stem Cells (Lonza) were cultured as per vendor's guidelines. Adipogenic and osteogenic differentiation kits were also purchased from Lonza. Oil-red-O stain (Millipore) was used to stain adipocytes and Vector Red (Vector Laboratories, Inc.) was used to stain osteocytes positive for alkaline phophatase activity. CellTrace™ CFSE fluorescent dye for proliferation assays was purchased from Invitrogen. hMSCs were cultured in either serum-containing growth medium (MSC-GM™ Lonza) or in a serum-free medium (BD Mosaic™ hMSC SF CCE). For serum-free culture, all vessels were coated with appropriate attachment factors. Cell growth and characterization studies using MSC-GM and BD Mosaic hMSC SF CCE media were conducted in independent laboratories to evaluate the performance of BD Falcon Multi-Flasks compared to control T-175 flasks.

Conclusions

- hMSC growth in media with and without serum was equivalent in BD Falcon Multi-Flasks and control T-175 flasks.
- hMSC morphology, attachment and proliferation rates were equivalent following culture in BD Falcon Multi-Flasks *vs.* T-175.
- hMSC cell expansion was higher and more rapid in BD Mosaic hMSC SF CCE media compared to serum containing media.
- Immunophenotype of late passage hMSCs were identical between cells grown in single and multi-layer vessels.
- The extent of differentiation observed with late passage hMSCs following culture in BD Falcon Multi-Flasks was comparable to cells grown in control T-175 flasks.
- BD Falcon Multi-Flasks provide a uniform culture environment to efficiently scale up stem cells as well as other diverse cell types from T-flasks without the need for lengthy protocol re-optimization.

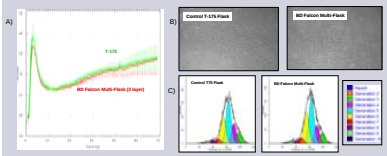
1 Equivalent Cell Growth and Cumulative Population Doublings of hMSCs In BD Falcon Multi-Flasks and Control T-175 Flasks





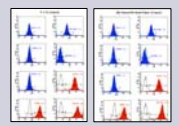
Cell yield per unit area and cumulative population doublings were equivalent for hMSCs grown in BD Falcon Multi-Flasks and T-175 flasks in media with and without serum. Four to five thousand cells/cm² were seeded in control T-175 and 3-layer BD Falcon Multi-Flasks in triplicate vessels and cultured in media with and without serum until approximately 80% confluence. Cells were harvested, counted and cell yield per unit area was determined. Cell yield per unit area is shown for hMSCs after passage 1 (left panel). Cumulative population doublings of hMSCs from each vessel type were calculated for five consecutive passages (right panel).

2 Equivalent Cell Morphology, Attachment and Proliferation from BD Falcon Multi-Flasks and Control T-175 Flask



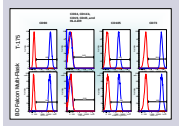
A) Cell attachment and growth profile of hMSCs were equivalent for cells cultured in BD Falcon Multi-Flasks and control T-175 flasks. Cell attachment, spreading and growth was measured using an impedance-based assay (xCELLigence™ RTCA MP System, Roche) following culture of hMSCs in MSC-GM for 5 days in each vessel type. Cells were plated in serum-free media on 96-well E-Plates and interactions between cells and electrodes, measured as changes in impedance, were reported as arbitrary cell index (CI) values. CI values are positively correlated with increased cell attachment and growth. **B) Morphology of hMSCs cultured in BD Falcon Multi-Flasks were indistinguishable from those cultured in control T-175 flasks. C) Proliferation profile of hMSCs were very similar for cells cultured in both single and multi-layered vessels.** hMSCs were stained with 2.5 mM carboxyfluorescein diacetate, succinimidyl ester (CFSE) for 10 minutes, washed and seeded in control T-75 and BD Falcon Multi-Flasks in MSC-GM. Five days after culture, cell proliferation was determined using flow cytometric analysis (FACS) and ModFit™ LT software (Verity Software House). ModFit LT's proliferation analysis of CFSE-stained cells shows distribution of cell populations in 10 generations of cell division. As cells divide, each daughter cell incorporates roughly half of the CFSE dye from its parent,allowing cell division to be tracked over time. The higher the generation of a cell the lower the CFSE fluorescence intensity (i.e., a shift to the left for the corresponding fluorescent histogram peak). The amplitude or height of each histogram represents the proportion (%) of cells in a given generation.

3 Immunophenotype of hMSCs Cultured in Media with Serum



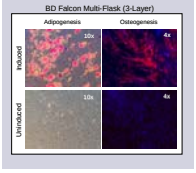
Immunophenotypic properties of hMSCs grown in BD Falcon Multi-Flasks for three consecutive passages in serum containing media were nearly identical to cells grown in parallel in T-175 control flasks. FACS analysis using BD FACSCalibur™ confirmed that hMSCs were negative (filled blue histograms) for surface markers CD14, CD34, CD45, CD79a and HLA-DR (BD Biosciences) and positive (filled red histograms) for expression of surface markers CD73, CD90 and CD105 (BD Biosciences). Respective isotype controls for each marker antibody are shown in grey (open histograms). Cells from three independent flasks of each type were pooled for FACS analysis.

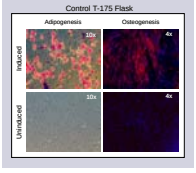
4 Immunophenotype of hMSCs Cultured in Serum-Free Media



Immunophenotypic properties of hMSCs grown in BD Falcon Multi-Flasks for three consecutive passages in serum-free BD Mosaic hMSC SF CCE media were also identical to cells grown in parallel in T-175 control flasks. Phenotype analysis was performed using BD Stemflow™ Human MSC Analysis Kit and run on BD LSRII using positive & negative cocktails in single tube format. hMSCs were positive for CD73, CD90 and CD105 and negative for CD34, CD11b, CD19, CD45 and HLA-DR. Isotype controls are shown in red and test samples are shown in blue. Cells from three independent flasks of each type were pooled for FACS analysis.

5 Multi-potency is maintained for hMSCs cultured in BD Falcon Multi-Flask





hMSCs grown for three consecutive passages in BD Falcon Multi-Flasks retained their multi-potent phenotype and were able to differentiate to adipocytes and osteocytes similar to cells grown in control T175 flasks. Post-differentiation, cultures were washed with 1X PBS and fixed with 4% paraformaldehyde. Cells induced for adipogenic differentiation were stained with Oil-Red-O (left panels). Intracellular lipid vacuoles (red) were observed in induced cells and were absent in un-induced control cells. Cells induced for osteogenic differentiation were stained with a substrate for alkaline phosphatase (red) and with a nuclear DAPI stain (blue) (right panel). Alkaline phosphatase positive cells were detected in induced cells but not in un-induced control cells.

6 Diverse Cell Types Can be Scaled-up Using BD Falcon Multi-Flasks

Cell Type	BD T-175 (cells/cm²)	BD Falcon Multi-flask (3-layer) (cells/cm²)	% of T-175
BHK-21	2.47×10^5	2.48×10^5	**100 %**
HUVEC-2	2.49×10^5	3.10×10^5	**125 %**
LnCap	9.95×10^4	9.92×10^4	**100 %**
Hep-G2	5.04×10^5	6.29×10^5	**104 %**
EcoPack™ 2-293 (ATCC 293 derived)	4.04×10^5	4.31×10^5	**107 %**
CHO-M1	2.49×10^5	2.31×10^5	**97 %**
MSC (adh tissue)	1.32×10^5	1.37×10^5	**104 %**
MSC (neuro-tissue)	2.88×10^5	3.23×10^5	**109 %**

Equivalent cell yield per unit area was observed with diverse cell types. Data shown is for 3-layer BD Falcon Multi-Flasks. Equivalent cell yield per unit area has also been observed for 5-layer BD Falcon Multi-Flasks (data not shown).