

Efficient Expansion of Human Mesenchymal Stem Cells (hMSCs) on Corning® Enhanced Attachment Microcarriers Using a Continuous Agitation Protocol

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Application Note

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Introduction

Microcarriers are small spheres used to culture adherent cells at scales that are impractical using traditional two-dimensional (2D) cultureware. Microcarriers maximize the surface area for adherent cell growth while minimizing the total culture volume and/or footprint. Microcarrier-based cultures, in which cells are grown on suspended microcarrier beads, can be maintained at small scale in spinner flasks (100 mL to 3L) or at large scale in stirred tank bioreactors (5L to 10,000L). Although 2D culture methods are well established for the majority of cell types, protocol development and optimization are required for microcarrier-based cell culture. Usually, optimization is accomplished at small scale in spinner flasks and utilizes offline measurements to monitor cell growth, gas levels, and nutrient exchange. Culture conditions are optimized to enable a uniform cell distribution on microcarriers with minimal shear while allowing sufficient exchange of nutrients and gases.

Corning has commercialized sterile, ready-to-use microcarriers with different surface chemistries for different cell types. Here we describe a protocol for human mesenchymal stem cell (hMSC) culture on Corning microcarriers in 1L glass spinner flasks. Two main challenges for microcarrier culture with hMSCs are: (1) these primary cells are sensitive to agitation, especially during cell attachment^{1,2}, and (2) they tend to form large cell-microcarrier aggregates during expansion^{3,4}. Our goal was to develop a continuous agitation protocol for efficient (>8-fold) expansion of hMSCs with minimal microcarrier clumping, while maintaining cell multipotency. The following parameters were evaluated: cell seeding density, microcarrier surface type, microcarrier concentration, agitation rate, working culture volume, and serum concentration during cell attachment. We achieved consistent hMSC yield of >40,000 cells per cm² (160,000 cells/mL) across multiple cell donors with minimal microcarrier clumping (2 to 3 beads/clump). Higher cell densities could be attained but with larger clump sizes (>5 beads/clump). After culture on microcarriers, cells retained typical spindle-like morphology, multipotent cell surface marker expression profile, and normal karyotype.

Materials and Methods

Cells

- ▶ Bone marrow-derived human mesenchymal stem cells (AllCells Cat. No. MSC-001F)

Microcarriers

- ▶ Corning® enhanced attachment microcarriers (Corning Cat. No. 3779)
- ▶ Corning Collagen-coated microcarriers (Corning Cat. No. 3786)
- ▶ 1L glass spinner flask (Corning Cat. No. 4500-1L), siliconized with Sigmacote® siliconizing reagent (Sigma Cat. No. SL2-100ML)

Vessels

- ▶ Corning CellBIND® surface 175 cm² angled neck cell culture flask with vent cap (Corning Cat. No. 3292)
- ▶ Corning CellBIND HYPERFlask® M cell culture vessel (Corning Cat. No. 10020)
- ▶ Corning CellBIND surface 6-well clear multiwell plates (Corning Cat. No. 3335)

Media and Reagents

- ▶ DMEM medium (Corning Cat. No. 10-013)
- ▶ MSC-qualified fetal bovine serum (Life Technologies Cat. No. 12662-011)
- ▶ Sterile, cell culture grade water (Corning Cat. No. 25-055-CM)
- ▶ DPBS (Dulbecco's phosphate buffered saline) (Corning Cat. No. 21-031-CV)
- ▶ TrypLE™ Select enzyme (1X) (Life Technologies Cat. No. 12563-011)

Consumables

- ▶ Corning 70 µm cell strainer (Corning Cat. No. 431751)
- ▶ Corning Easy Grip polystyrene storage bottles (Corning Cat. Nos. 431175, 430282)
- ▶ Stripette™ serological pipets (Corning Cat. Nos. 4487, 4488, 4489, 4490, 4491)
- ▶ Falcon® 50 mL high clarity PP centrifuge tube (Corning Cat. No. 352070)

Lab Equipment

- ▶ Wheaton Micro-Stir® magnetic stirrer (VWR Cat. No. 12000-908)
- ▶ Vi-CELL® Analyzer (Beckman Coulter Cat. No. 731050)

hMSC Scale-up from Thaw

hMSCs (designated as passage 1) were thawed from a cryogenic storage vial and seeded at 5,000 cells/cm² on Corning® CellBIND® surface T-175 flasks in 10% hMSC-qualified FBS/DMEM.

NOTE: the cell seeding density of 5,000 cells/cm² was selected for optimal passage [e.g., every 5 days]. Seeding density should be optimized for different cell donors and/or expansion rate.

Cells were sub-cultured after 5 days when 75% confluent (~20,000 cells/cm²) and seeded at 5,000 cells/cm² on Corning CellBIND surface HYPERFlask® vessels (passage 2). At 75% confluence, cells were harvested (passage 3) and seeded onto microcarriers (1,600 cm² per 1L siliconized glass spinner flask, 11 g/L) at 6,000 cells/cm² in a 400 mL final volume.

NOTE: a slightly higher seeding density may improve cell attachment when transitioning from 2D to microcarrier cultures.

Cell Attachment and Expansion on Microcarriers

Cell attachment to microcarriers occurred under constant agitation at 15 rpm for 20 hours. After the attachment phase, the agitation was increased to 30 rpm. Microcarrier clumping was monitored daily, and the agitation was increased by 10 rpm every 2 days starting on day 3 to prevent microcarrier clumping. The maximum agitation rate was 80 rpm. Half-volume medium changes were performed on day 3 and then every 2 days thereafter.

Cell Counts

For daily assessments of cell growth, 5 mL samples were collected, and cells were harvested from microcarriers via a 5-minute TrypLE™ treatment followed by filtration through a 70 µm cell strainer. Cell number and viability were assessed using an automated Vi-CELL analyzer.

Cell Characterization

Morphology

To assess cell morphology after microcarrier culture, cells were removed from microcarriers using TrypLE and re-seeded onto Corning CellBIND surface 6-well plates.

hMSC Surface Markers

Cells were removed from microcarriers using TrypLE, washed with PBS, and fixed in 4% paraformaldehyde. Quantitative phenotypic marker expression was evaluated for CD73, CD90, CD105, CD14, and CD34 via standard immunofluorescent staining and flow cytometry.

Karyotype

Cells were removed from microcarriers using TrypLE and re-seeded into Corning CellBIND surface T-75 flasks for karyotype analysis at WiCell Research Institute.

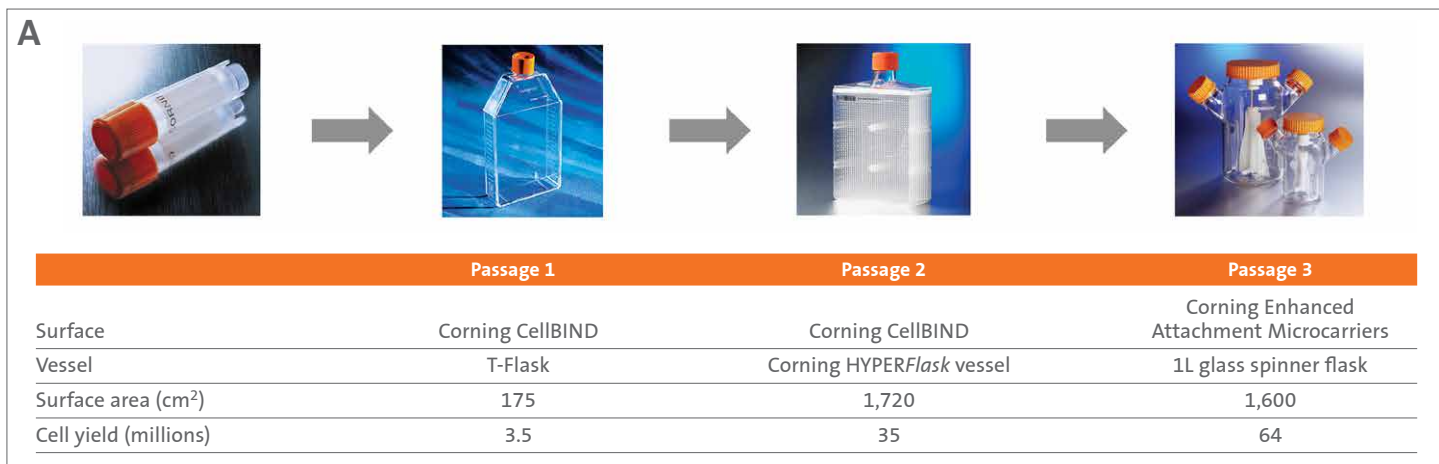


Figure 1A. hMSC scale-up for glass spinner flask inoculum.

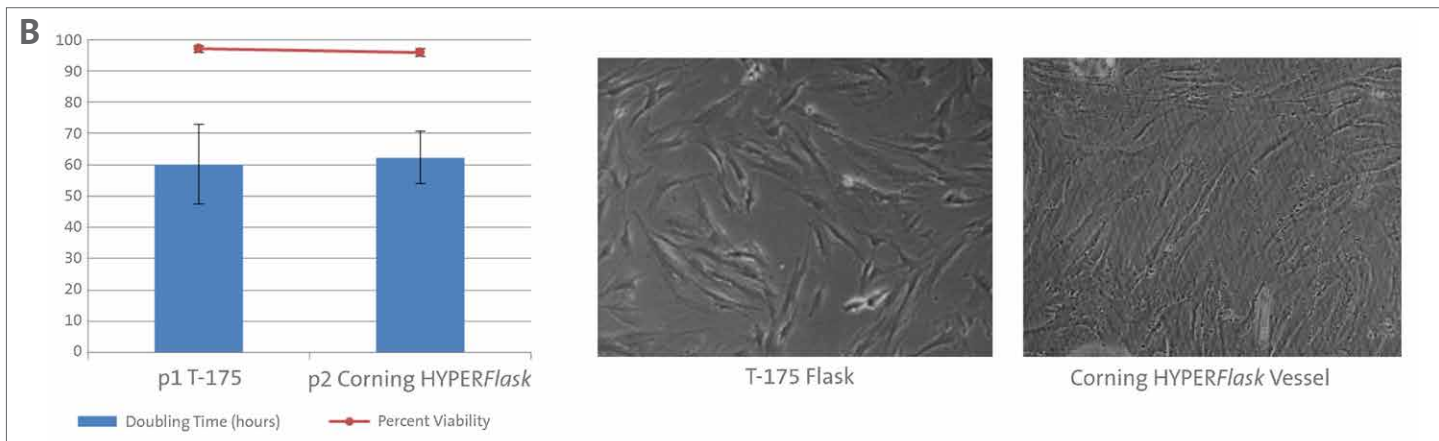


Figure 1B. No significant change in cell morphology, doubling time, or viability was observed for hMSC scale-up.

Results and Discussion

hMSC Expansion Protocol to Generate Sufficient Number of Cells for Microcarrier Inoculum

For microcarrier culture at liter(s) scale, 20 to 100 million cells are required for cell seeding. Due to limited hMSC yields at isolation, an efficient and consistent method is required to generate a large number of cells for a microcarrier inoculum. Here we describe a cell scale-up protocol using Corning HYPERFlask vessels. As shown in Figure 1A, passage 1 cells were thawed into T-175 flasks and then passaged to HYPERFlask vessels before being seeded in glass spinner flasks. This scale-up method was evaluated twice for 3 bone marrow donors, and no significant change in cell morphology, doubling time, or viability was observed (Figure 1B, error bars represent $n = 6$). This method was used to generate the cell inoculum required for the optimization of microcarrier culture conditions in 1L glass spinner flasks.

Continuous Agitation Protocol for Attachment and Expansion of hMSC on Corning Microcarriers in 1L Glass Spinner Flasks

To determine optimal culture conditions, cells were seeded on microcarriers in 1L glass spinner flasks (Figure 2A). Cultures were expanded for 9 to 11 days, after which cells were evaluated for

percent viability, total yield, and retention of typical morphology, surface marker expression, and normal karyotype. The following culture parameters were evaluated during protocol optimization: cell seeding density, microcarrier surface type, microcarrier concentration, agitation rate, working culture volume, and serum concentration during attachment. Using the optimized parameters shown in Figure 2B, cells were expanded on microcarriers under continuous agitation in glass spinner flasks. Cell yields greater than 50,000 cells/cm² (200,000 cells/mL) were achieved while maintaining high cell viability (Figure 3A). Duplicate spinner flask cultures were evaluated for each microcarrier type. Small microcarrier clump size (2 to 3 microcarriers per clump) was maintained with Corning® enhanced attachment microcarriers by increasing the agitation rate after medium re-feeds (Figure 3B, phase images). For Corning Collagen-coated microcarriers, it was more difficult to maintain small clump size with agitation, resulting in sampling error. Therefore, there are larger error bars starting at day 6 (Figures 3A graph and 3B phase images). Day 7 cells were stained with a viability stain, Calcein AM, to better visualize cell morphology, microcarrier coverage, and microcarrier clump size (Figure 3B, FITC images).

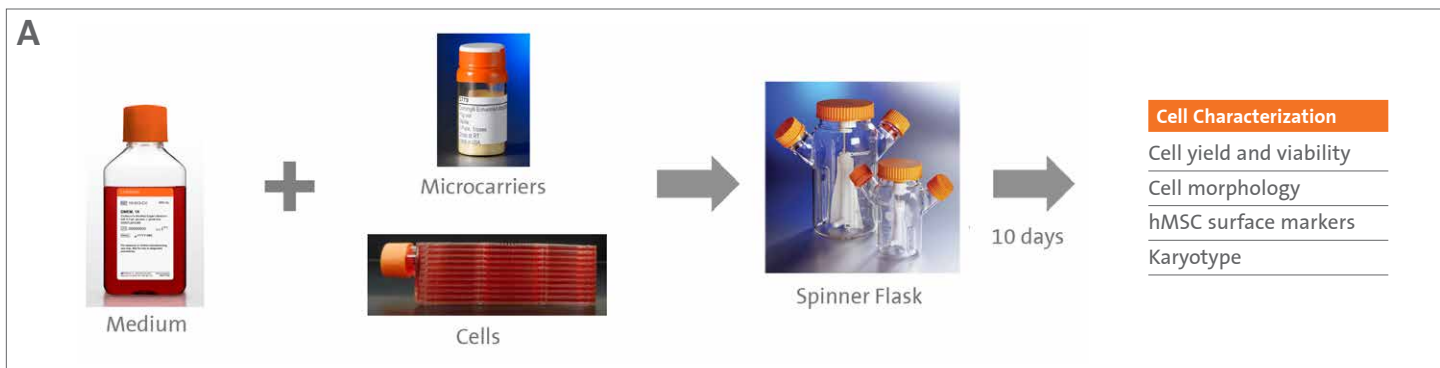


Figure 2A. Experimental workflow for optimization of microcarrier culture conditions

B

| Attachment | |
|---|--|
| Cell seeding density | 6,000 cells per cm ² |
| Serum concentration | 10% |
| Microcarrier surface | Enhanced attachment |
| Microcarrier concentration | 4 cm ² /mL (11 g/L) |
| Agitation rate | Continuous at 15 rpm for 20 hours |
| Working culture volume | 400 mL |
| Vessel format | 1L glass spinner flask |
| Expansion | |
| Replenish medium | 50% volume; days 3, 5, 7, 9, and 11 |
| Agitation rate | Continuous at 30 rpm to 80 rpm |
| Microcarrier clump size | 2 to 3 microcarriers/clump |
| Agitation rate to control microcarrier clump size | Increase by 10 rpm on day of re-feed |
| Duration of culture | 10 days (>40,000 cells/cm ²) |

Figure 2B. Optimized conditions for hMSC attachment and expansion on Corning enhanced attachment microcarriers in 1L glass spinner flask.

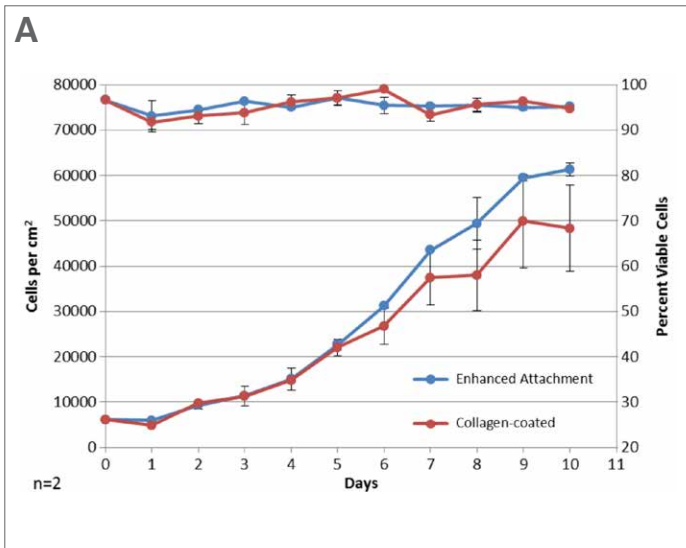


Figure 3A. Efficient hMSC expansion on Corning microcarriers using an optimized continuous agitation protocol.

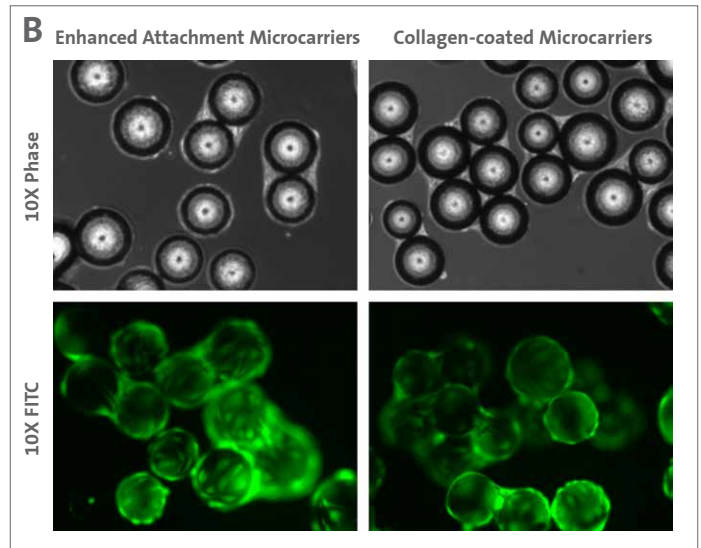


Figure 3B. Small cluster size and standard hMSC morphology were maintained on Corning microcarriers.

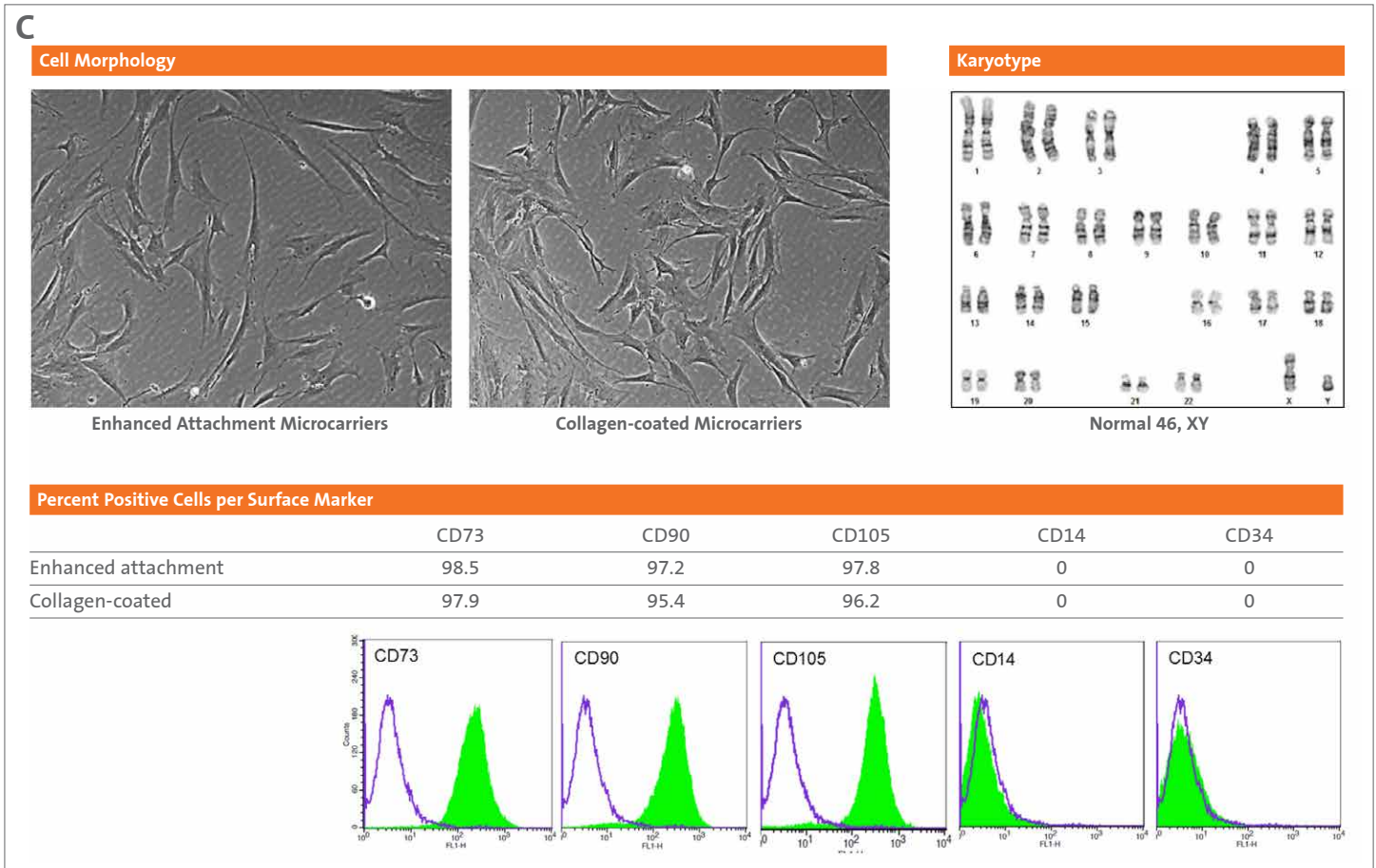


Figure 3C. hMSCs retained typical morphology, marker expression, and normal karyotype after culture on microcarriers.

After 10 days, cells were removed from microcarriers using TrypLE™ and re-seeded onto Corning® CellBIND® surface 6-well plates at 5,000 cells/cm². As shown in Figure 3C, cells retained typical elongated morphology, normal 46, XY karyotype, and high expression of hMSC surface markers, CD73, CD90, and CD105, after culture on enhanced attachment and Collagen-coated microcarriers.

Validation of the Continuous Agitation Protocol Across Multiple hMSC Donors

hMSCs isolated from different bone marrow donors often have different recovery and expansion rates after cryopreservation. To test the robustness of our protocol, hMSCs from 3 bone marrow donors were thawed and expanded on Corning enhanced attachment microcarriers as shown in Figure 1A (scale-up) and Figure 2B (microcarrier culture conditions). For each hMSC donor, two independent experiments were performed from thaw to microcarrier expansion. As shown in Figure 4, high cell yields (>40,000 cells/cm²) were observed for all donors, confirming the robustness of this protocol. As anticipated with the variability in hMSCs from different donors, a higher rate of cell expansion was observed for donor 4880 compared to donors 4853 and 4415.

Conclusions

- ▶ hMSCs attached to and expanded on Corning enhanced attachment microcarriers using continuous agitation in 1L glass spinner flasks (from 6,000 cells/cm² to >40,000 cells/cm² in 10 days).
- ▶ Microcarrier clump size was maintained at <5 microcarriers per clump by increasing the agitation rate after culture re-feeds every 2 to 3 days.
- ▶ hMSCs cultured on Corning enhanced attachment microcarriers maintain standard morphology, a typical phenotypic marker expression profile, and normal karyotype.

References

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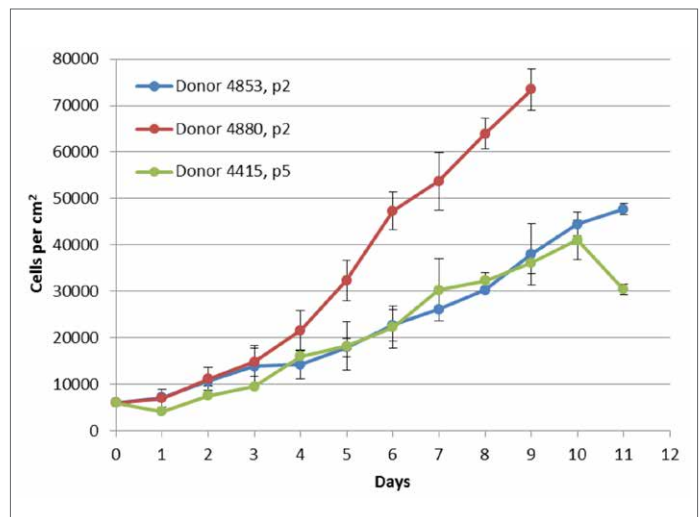


Figure 4. Protocol consistency was demonstrated across three hMSC bone marrow donors.

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