Efficient Expansion of Human Mesenchymal Stem Cells on Synthetic Microcarriers in Serum-Free, Defined Medium Using Bead-to-Bead Transfer

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Introduction

Cell-based therapies require efficient production of a consistent cell product in large quantities. Microcarriers provide a three-dimensional culture environment that enables rapid cell expansion in dynamic conditions. Traditional microcarriers generally require time-consuming and labor-intensive preparation that can affect the consistency and efficiency of cell production. Further, serum-containing medium may be required to facilitate cell attachment and expansion in dynamic conditions. These limitations hinder the use of microcarriers for cellular therapeutics and biopharmaceutical manufacturing.

Here we report new synthetic, ready-to-use microcarriers for scalable cell production in dynamic conditions. These microcarriers were used to expand human bone marrow-derived mesenchymal stem cells (hMSCs) for multiple passages in animal component-free (ACF), defined medium (stemgro hMSC medium). We demonstrate cell expansion by bead-to-bead transfer without the need for enzymatic dissociation. Cells retained typical spindle-like morphology, cell surface marker expression profile, multipotency, and normal karyotype. Further, we demonstrate short-term expansion on synthetic microcarriers with 3 hMSC donors, with several commercially-available media, and with 125 mL to 3L disposable spinner flasks.

Methods

Cells:

- Human bone marrow-derived mesenchymal stem cells
  STEMCELL™ Technologies, Cat. No. MSC-001F
  Lonza, Cat. No. PT-2501

Media:

- Corning® stemgro® hMSC Medium
  Corning®, Cat. No. 40-410-KIT
- Serum-containing Medium
  IMDM, Corning, Cat. No. 10-016-CV
  MSC-qualified FBS, Life Technologies, Cat. No. 12662-002

Microcarriers/vessels:

- Corning® Synthemax® II microcarriers
  Corning, Cat. No. 3781
- Enhanced attachment microcarriers
  Corning, Cat. No. 3779
- Polystyrene microcarriers
  Solohill Engineering, Cat. No. P102-1521
- Cytodex® 1 microcarriers
  GE Healthcare, Cat. No. 17-0448-01
- Disposable spinner flasks
  Corning, Cat. No. 3152, 3561, 3563

hMSC thaw onto microcarriers:

hMSCs from a working cell bank were removed from liquid nitrogen storage and thawed in a 37°C water bath. Cells were pelleted to remove DMSO freezing medium, reconstituted in pre-warmed Corning stemgro hMSC medium, and seeded directly onto microcarriers (150 cm^2 per spinner flask) at 5000 cells per cm^2 in a 15 mL final volume. Cell adhesion occurred in static conditions for 18-20 hours. After that, medium volume was adjusted to 45 mL, and cultures were agitated at 30 rpm for 15 minutes every 2 hours. On day 3, 50% of the culture medium was replenished, and cells were first passaged on day 4 using the dilution protocol described below.

hMSC expansion on microcarriers:

hMSC viability and number were assessed at the end of each passage, by collecting 3 mL culture samples. Cells were removed from microcarriers with 3-5 minutes trypsin/EDTA or trypLE treatment and then strained through a 70 micron filter to remove microcarriers prior to cell count with an automated cell number/viability analyzer, Vi-Cell™.

For passaging, hMSC-microcarrier cultures were diluted with fresh microcarriers (150 cm^2 per spinner flask) to obtain a density of 3500-5000 cells per cm^2 in a final volume of 15 mL. After 18-20 hours of adhesion under static conditions, medium volume was adjusted to 45 mL, and cultures were agitated at 30 rpm every 2 hours for 15 minutes. Culture medium (50%) was replenished every 3 days.

Expression of hMSC markers (positive: CD73, CD90, and CD105; negative CD14 and CD45) was assessed by flow cytometry at passage 5.

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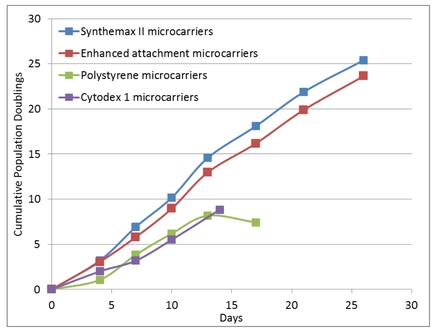
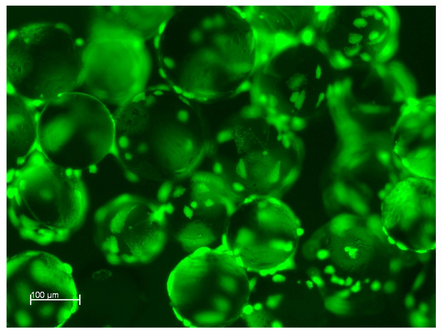
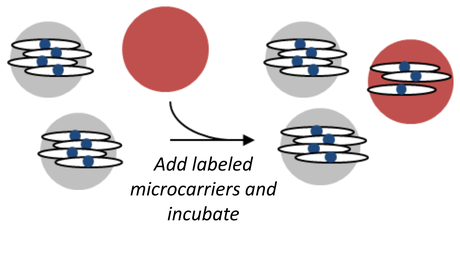
Figure 1: Efficient hMSC recovery and expansion on synthetic microcarriers in animal component-free (ACF), defined Corning stemgro hMSC medium.

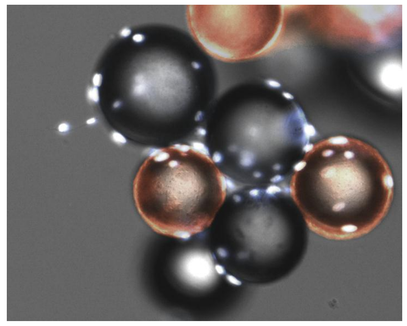




hMSCs (STEMCELL™ Technologies) were thawed and expanded on microcarriers under intermittent agitation in spinner flasks for 26 days. Cells were counted at each passage (individual data points on the graphs), and based upon cell count, ~90% of the culture was removed prior to adding fresh microcarriers to obtain a density of 3500-5000 cells per cm^2. No enzyme dissociation was required for cell migration and attachment to fresh microcarriers. Cumulative population doublings (left) are shown for hMSCs thawed and expanded on microcarriers in Corning stemgro hMSC medium. In 26 days, Corning microcarriers supported ~25 population doublings, resulting in a net projected cell yield of ~200 billion cells in ACF, defined medium. A representative image of Calcein AM-stained hMSCs on Enhanced attachment microcarriers is shown (right). Uniform cell confluence (80-90%) was observed on synthetic microcarriers after 4-5 days in culture.

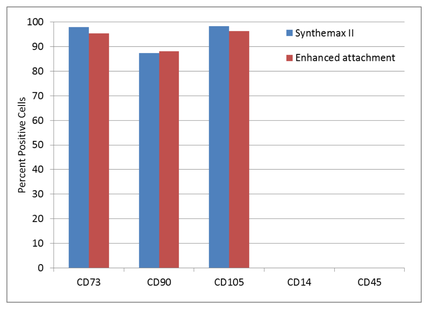
Figure 2: Bead-to-bead transfer of hMSCs on synthetic microcarriers in ACF Corning stemgro hMSC medium.

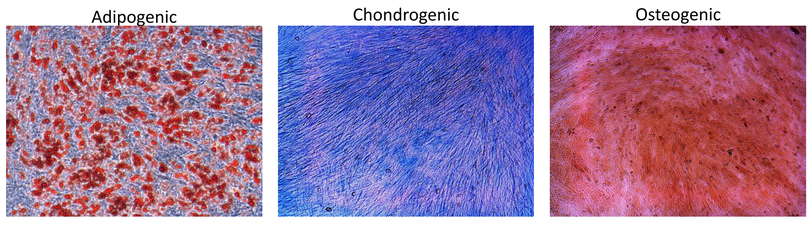




Cells were seeded onto Corning Synthemax II microcarriers in spinner flasks, as described in Methods. On day 2, rhodamine-labeled Synthemax II microcarriers were added to the culture as shown (left). After 24 hours of intermittent agitation, cells were stained with nuclear dye, DAPI, and imaged on microcarriers using fluorescence microscopy. Cells colonized the rhodamine-labeled microcarriers (red), indicating that cells migrated to new microcarriers with intermittent agitation (right).

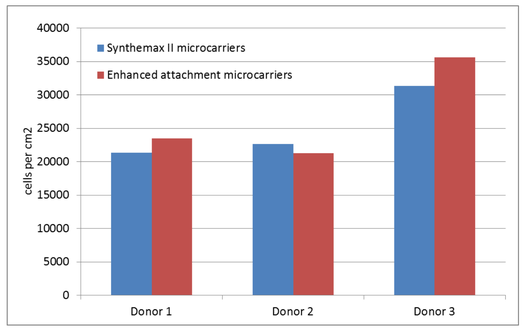
Figure 3: Normal hMSC immunophenotype and trilineage differentiation potential were retained after 5 passages on synthetic microcarriers in ACF Corning stemgro hMSC medium.





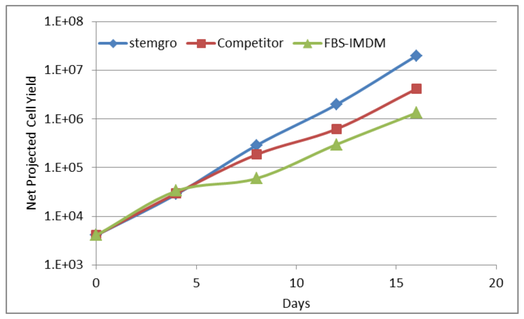
For each condition, passage 5 cells were removed from microcarriers using trypsin-EDTA, and microcarriers were separated using a 70 micron cell strainer. Cells were fixed in paraformaldehyde and stained for specific phenotypic markers prior to flow cytometry analysis. Quantitative phenotypic marker expression for hMSCs maintained on Corning Synthemax II and Enhanced attachment microcarriers are shown (top). To demonstrate cells retain their trilineage differentiation potential, cells from each microcarrier condition were replated on Corning® CellBIND® 6 well plates. According to manufacturers' protocols, adipogenic (hMSC Differentiation BulletKit®-Adipogenic kit; Lonza, Cat. No. PT-3004), chondrogenic (STEMPRO® Chondrogenesis Differentiation Kit; Gibco, Cat. No. A10071-01) and osteogenic (MesenCult® Osteogenic Stimulatory Kit; Stem Cell Technologies, Cat. No. 05404) differentiations were maintained for ~20 days before cells were fixed and stained with Oil Red O (adipocytes), Alcian Blue (chondrocytes), or Alizarin Red (osteocytes). Representative images are shown (bottom). In addition, hMSCs cultured on Corning microcarriers in Corning stemgro hMSC medium retained normal 46,XY karyotype (data not shown). G-banded karyotype analysis was performed by WiCell Cytogenetics Lab for each cell-microcarrier condition.

Figure 4: Cell expansion on synthetic microcarriers in ACF Corning stemgro hMSC medium is confirmed for 3 hMSC donors.



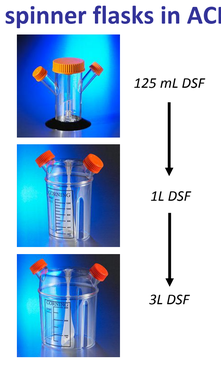
hMSCs from 3 donors were expanded on microcarriers under intermittent agitation in spinner flasks for one passage in Corning stemgro hMSC medium. For each donor, hMSCs were thawed directly from the vendor vial into Corning stemgro hMSC medium in Corning CellBIND® T75 flasks. Cells were allowed to reach 80% confluence and subcultured at 3500 cells per cm^2 onto synthetic microcarriers. After 4 days, cells were harvested from microcarriers using trypLE, and the number of cells per cm^2 was calculated. Donor 1 and 2 are from STEMCELL™ Technologies. Donor 3 is from Lonza.

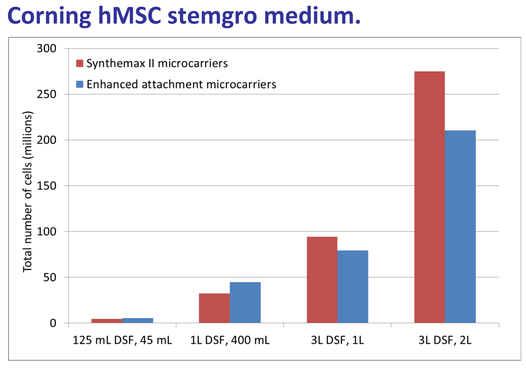
Figure 5: hMSC expansion on synthetic microcarriers in different media.



hMSCs (Lonza) were thawed and expanded on Corning Synthemax II microcarriers in 3 different media under intermittent agitation in spinner flasks. Cells were counted at each passage (individual data points on the graphs), and based upon cell count, ~90% of the culture was removed prior to adding fresh microcarriers to obtain a density of 3500-5000 cells per cm^2. Net projected cell yield is shown for hMSCs thawed and expanded on microcarriers in Corning stemgro hMSC medium (blue), 10% FBS-IMDM (green), and competitor serum-free, defined medium (red) for 4 passages.

Figure 6: hMSC scale-up on synthetic microcarriers in disposable spinner flasks in ACF Corning hMSC stemgro medium.





hMSCs (Lonza) were expanded on synthetic microcarriers under intermittent agitation in disposable spinner flasks (DSF). Cells were seeded at 3500 cells per cm^2 in 125 mL DSF with a final volume of 45 mL Corning stemgro hMSC medium (3.3 cm^2 microcarriers per mL). After 4 days, the culture was used to seed a 400 mL culture in a 1L DSF. The remaining passages in 3L DSF were performed similarly, maintaining 3.3 cm^2 microcarriers per mL. We achieved 400-500 fold expansion in 18 days on synthetic microcarriers in ACF medium.

Conclusions

- hMSCs were successfully maintained for 7 sequential passages on sterile, ready-to-use Corning microcarriers in ACF Corning stemgro hMSC medium in spinner flasks, resulting in a net projected yield of ~200 billion cells.
- Corning synthetic microcarriers enabled bead-to-bead transfer of hMSCs without the need for enzymatic cell dissociation.
- hMSCs cultured on Corning synthetic microcarriers in Corning stemgro hMSC medium maintained normal phenotypic marker expression profile, trilineage differentiation potential, and karyotype.
- Cell expansion on Corning synthetic microcarriers was confirmed for 3 hMSC donors and with 3 commercially-available media.
- We demonstrated efficient hMSC expansion to 3L spinner flasks on Corning synthetic microcarriers in Corning hMSC stemgro medium.