Long term culture of human mesenchymal stem cells on Corning® Synthemax® II microcarriers in xeno-free conditions

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Abstract: Large scale production of human Mesenchymal Stem Cells (hMSCs) for cell therapy faces several challenges, including the requirement of large surface area and defined, xeno-free culture conditions. Microcarrier technology provides an effective solution for large scale cell production.
Here we report the long term culture of human bone marrow-derived MSCs on Corning Synthemax II synthetic microcarriers in xeno-free medium, in disposable spinner flasks. Cells were maintained for 7 weeks on microcarriers under agitated conditions and population scale-up was obtained by addition of fresh beads and spontaneous bead-to-bead transfer. Under these conditions, cells expanded 10,000-fold while maintaining their immunophenotype and tri-lineage differentiation potential. To our knowledge, this report is the first example of long term culture of hMSCs on synthetic microcarriers in xeno-free conditions.

Introduction

Production of hMSCs for cell therapy faces two major challenges: the necessity to grow cells in completely defined conditions (medium and substrate) to avoid any potential contamination and a culture method allowing the generation of very large quantities of cells required for this type of application (up to 5.10^8 cells/dose).
Today several defined xeno-free media are commercially available and provide good performance for hMSCs culture. With regard to a substrate, commercial synthetic surface, such as Corning Synthemax Surface has been validated for the production of stem cells in T75 and T225 flasks. For larger scale cultures, microcarriers used in stirred reactor remain a technology of choice. Completely synthetic microcarriers that could support the expansion of stem cells in serum-free defined media would represent an ideal substrate for the scalable production of stem cells for cell therapy.
In this poster we describe the method developed to culture hMSCs on synthetic Corning Synthemax II microcarriers in xeno-free medium, under stirred conditions. With this method, we demonstrated the possibility of spontaneous bead to bead transfer of hMSCs to scale up the cell population. We were able to achieve a cumulative hMSCs expansion of 10,000 fold while maintaining cell immunophenotype and multipotency. We believe this method of large scale production of hMSCs under xeno-free defined culture conditions will be of interest for production of clinical grade cell for therapeutic applications.

Material and methods

Cells:
Human bone marrow-derived mesenchymal stem cells (Cat. No. MSC-001F) were maintained in Mesencult®-XF medium (Cat. No. 05429). For regular 2D cultures cells were grown on Mesencult®-XF attachment substrate coated plates (Cat. No. 05424) all from STEMCELL Technologies.

Spinner flasks cultures:
500 mg of gamma sterilized Synthemax II polystyrene beads (Corning, Cat No. 3781XXS1), corresponding to 150 cm2 were suspended in Mesencult®-XF medium and transferred to 125 mL Corning® spinner flasks (Corning, Cat. No. 3152). About 750,000 cells were added to the spinner flasks and the volume of medium was adjusted to 15 mL. Cells were placed in a cell culture incubator without agitation for 16 hours, then the volume of medium was adjusted to 30 mL and the cultures were stirred at 30 rpm for 15 minutes every 2 hours. Twenty mL of medium was replaced every other day.
Every week, 75% of the culture was removed after gentle homogenization and fresh beads were added to the spinner flask. Collected beads were treated with trypsin and detached cells were counted and used for flow cytometry analysis or differentiation experiments.

Flow cytometry:
Flow cytometry analysis were performed with a BD FACSCalibur®. The results were acquired and analyzed using Cellquest® Pro software.

Directed differentiation:
The differentiations were induced using the following kits and manufacturer's instructions:
- Adipogenic differentiation kit (Lonza, Cat. PT3102A/B).
- StemPro® Osteogenic differentiation kit (Gibco, Cat. No. A10072-01).
- StemPro® Chondrogenic differentiation kit (Gibco, Cat. No. A10071-01).

Results

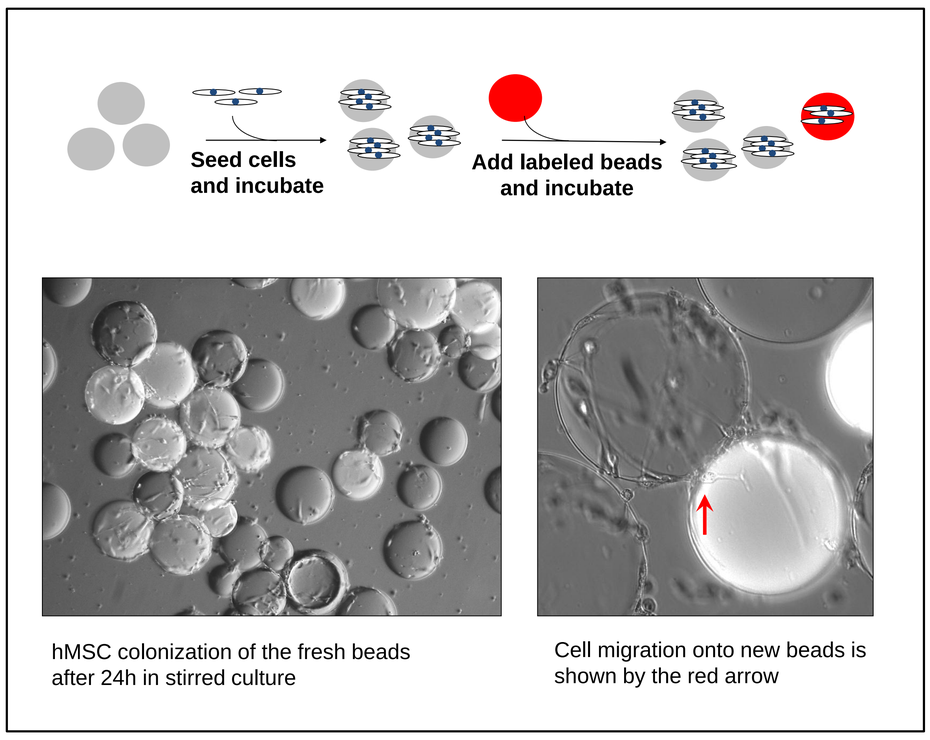


Figure 1: Spontaneous bead-to-bead transfer. As illustrated on the drawing, the cells were cultured on Corning beads, then labeled fresh beads were added to the culture. The fluorescent microscopy pictures illustrate the colonization of new beads (lighter color) by hMSCs after 24 hours of culture. Cell migration during bead to bead contact (red arrow) seems to be an important part of this process.

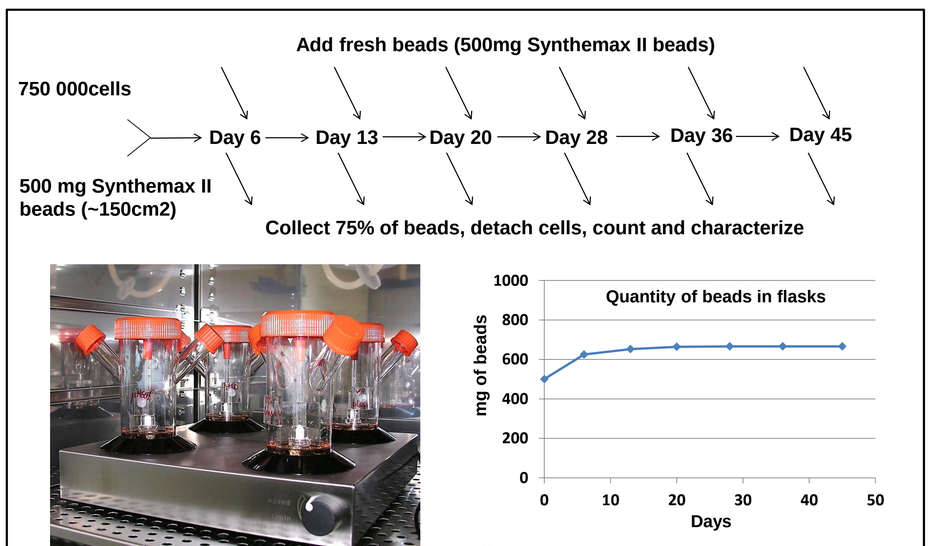


Figure 2 : Long term expansion protocol. Cells were seeded on microcarriers and cultured in spinner flasks. Every week, 75% of the culture was collected for counting and characterization and fresh beads were added to the remaining culture.

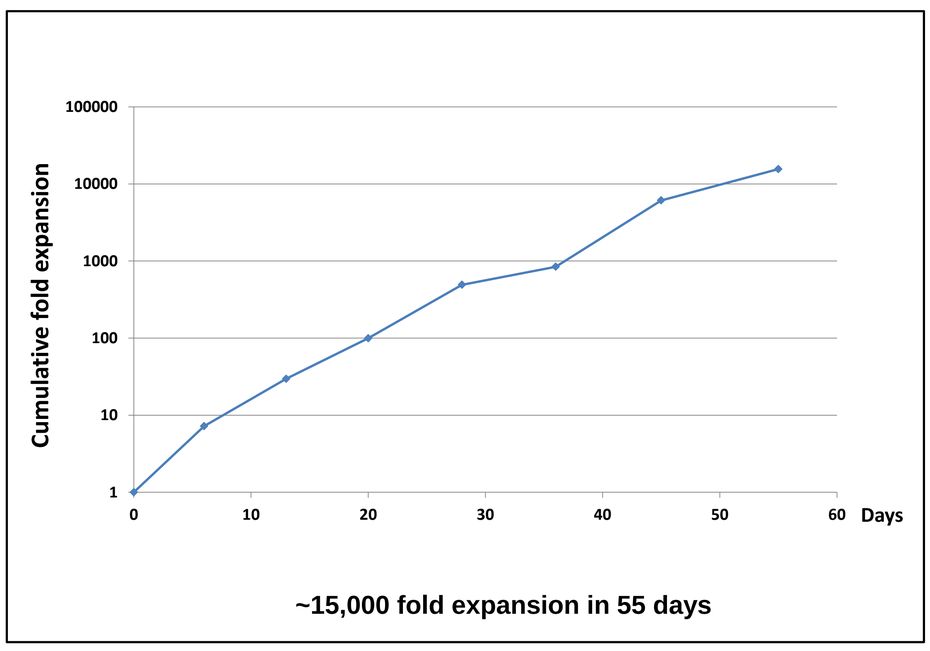
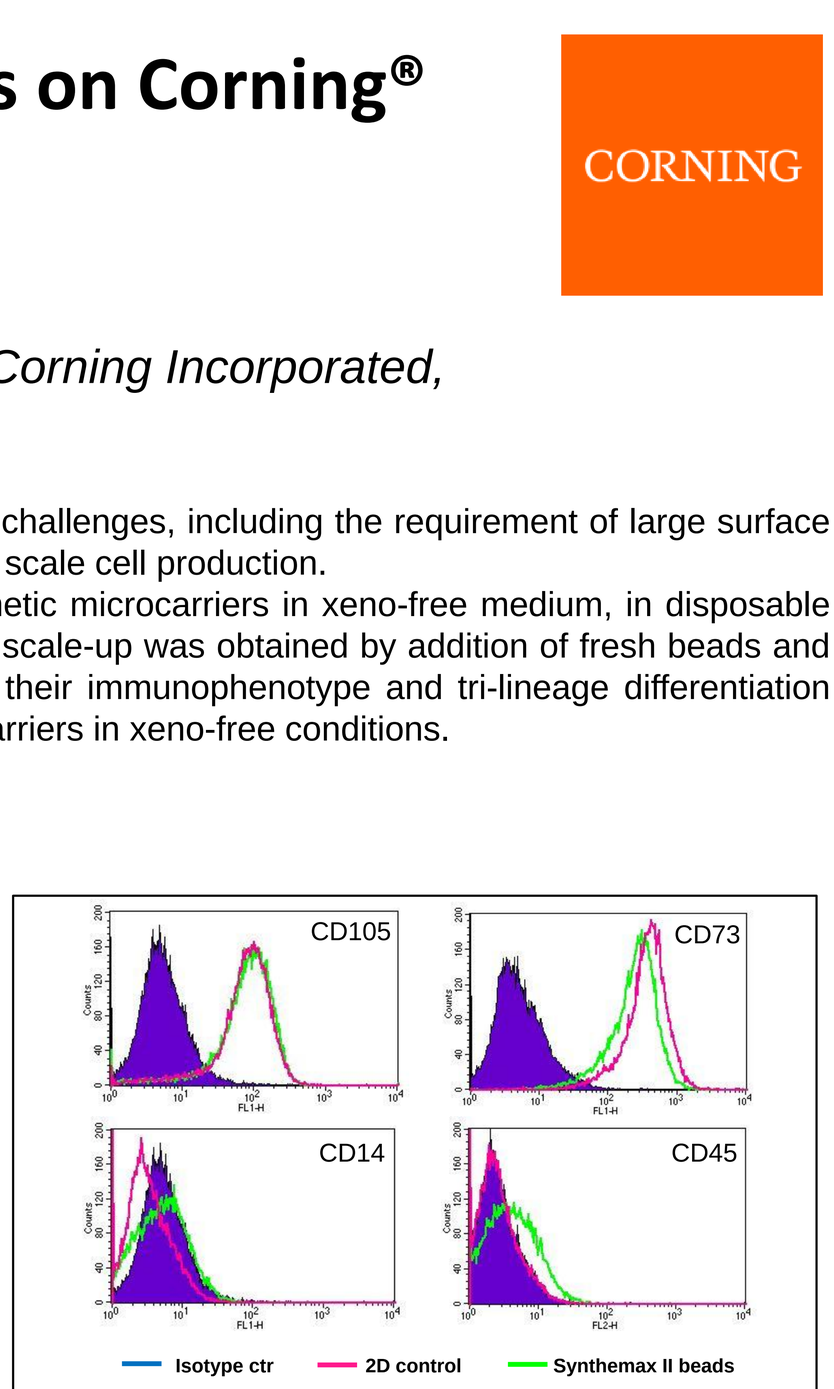


Figure 3: Long term hMSC expansion on Corning Synthemax II microcarriers. Cells were counted at every passage, and the cumulative cell expansion was calculated.



	CD105	CD73	CD14	CD45
2D control	91%	99%	<1%	<1%
Synthemax II beads	91%	97%	<1%	<1%

Figure 4: Phenotypic characterization of hMSCs after 7 weeks of culture on Corning Synthemax II microcarriers. The expression of all four markers was comparable for cells grown on microcarriers and 2D conditions.

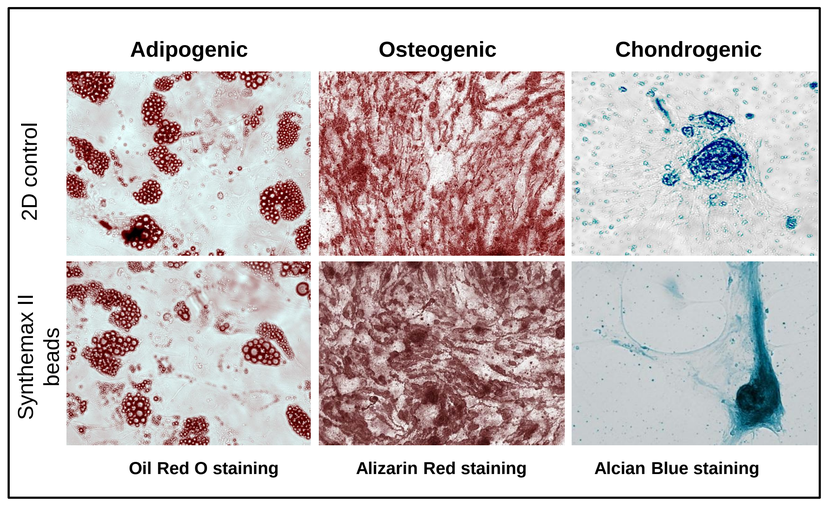


Figure 5 : Directed differentiation of hMSCs after long-term expansion on Corning Synthemax II microcarriers. Cells grown for 7 weeks on microcarriers were induced to adipogenic, osteogenic and chondrogenic differentiations. Two weeks after induction, cells were fixed and stained with Oil Red O, Alizarin Red and Alcian Blue, respectively.

Conclusions

-Corning Synthemax II microcarriers are synthetic, ready-to-use microcarriers that enable long term-expansion of hMSCs in xeno-free defined medium.

-Corning Synthemax II microcarriers allow bead-to-bead transfer of hMSCs, an important process for easy, enzyme-free cell expansion.

-hMSCs grown on Corning Synthemax II microcarriers for 7 weeks maintained typical phenotype and the ability for tri-lineage differentiation.

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