A New Microcarrier Platform for Efficient Cell Production and Recovery

Jennifer Weber[1], Martial Hervy[2], Marylene Pecheul[2], Yue Zhou[1], Corinne Walerack[2], David Henry[2], Marie Maud Bear[2], and Stephen Caracci[1]

[1]One Science Center Drive, Corning Incorporated, Corning Life Sciences, Corning, NY 14831 USA
[2]Corning European Technology Center, 77210 Avon, France

Introduction

Cell culture on microcarriers is an increasingly important technique for the production of large cell quantities. Using this method, cell harvest, separation, and concentration processes can be time and resource consuming. To address this issue, we have developed a new generation of microcarriers that can be quickly dissolved (with protease-free, animal-free reagents), allowing easy collection of the grown cells without the need for microcarrier separation.

We present potential applications for dissolvable microcarriers. Human mesenchymal stem cells (hMSCs), MRC5, and Vero cells were grown in spinner flask cultures on dissolvable microcarriers. For all cell types evaluated, growth performance on dissolvable microcarriers was equivalent to polystyrene microcarriers.

After multi-passage growth on microcarriers using bead-to-bead transfer, hMSCs retained phenotypic marker expression and differentiation capability. Alternatively, we show that cells harvested via bead dissolving can efficiently attach and grow on fresh microcarriers.

The use of dissolvable microcarriers presented here demonstrates the efficient production and recovery of high quality product without the need for microcarrier separation.

Methods and Materials

Microcarriers:

- Corning® Enhanced attachment microcarriers (Corning Cat. No. 3779)
- Corning Synthemax® II polystyrene microcarriers (Corning Cat. No. 3781)
- Cytodex 3 (GE Healthcare Cat. No. 17-0485)
- Dissolvable microcarriers coated with Corning Synthemax II or denatured Collagen

Cell culture on microcarriers:

- hMSCs were seeded onto microcarriers (200 cm^2) in spinner flasks (Corning Cat. No. 3152) at 5,000 cells per cm^2 in 10 mL of MesenCult™-XF medium. Cells were maintained under static conditions for the first 18 hours. The culture volume was then adjusted to 40 mL and intermittent agitation (15 minutes every 2 hours at 30 rpm) was applied. Culture medium (75% volume) was exchanged on day 4. Cultures were maintained in a humidified, 5% CO_2 incubator.
- MRC5 and Vero cells were seeded onto microcarriers (200 cm^2 per spinner flask) at 5,000 cells per cm^2 in 40 mL of IMDM + 10% FBS. Cells were maintained under static conditions for the first 2 hours, then continuous agitation at 30 rpm was applied. For MRC5, a 75% volume medium change was performed on day 4. Cultures were maintained in a humidified, 5% CO_2 incubator.

Conclusions

- Corning developed a new dissolvable microcarrier platform which allows easy cell recovery with protease-free, animal-free reagents.
- MRC5 and Vero cell growth on denatured Collagen dissolvable microcarriers and hMSC growth on Synthemax II dissolvable microcarriers were comparable to polystyrene and/or Cytodex 3 controls.
- Further, we demonstrated that Synthemax II dissolvable microcarriers support hMSC expansion in two commercially available xeno-free media, and cells were expanded using serial passage by bead-to-bead transfer for 40 days, achieving a cumulative growth of 10,000-fold.
- After long-term culture on Synthemax II dissolvable microcarriers, hMSCs retained their standard phenotypic marker expression profile and the ability to undergo directed differentiation.
- Lastly, hMSCs harvested via bead dissolving efficiently attached and grew on fresh microcarriers.

Results

Figure 1.

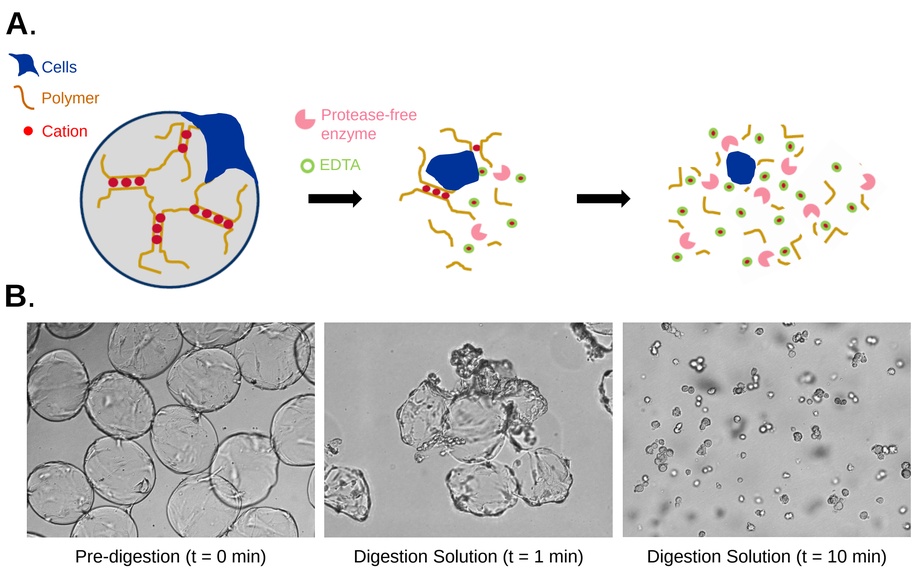


Figure 1. Cell harvest from dissolvable microcarriers results in complete digestion of microcarriers. (**A**) A schematic representation of bead digestion over time is shown. Cells adhere to a peptide- or protein-coated ionically cross-linked polysaccharide microcarrier. Chelation of divalent cations by EDTA destabilizes the polymer crosslinking and exposes the polymer chains to degradation by a protease-free enzyme. After 5 to 10 minutes, the microcarriers are completely dissolved, and cells are released in the medium. (**B**) Phase contrast microscopy images are shown of the bead digestion and subsequent cell release.

Figure 2.

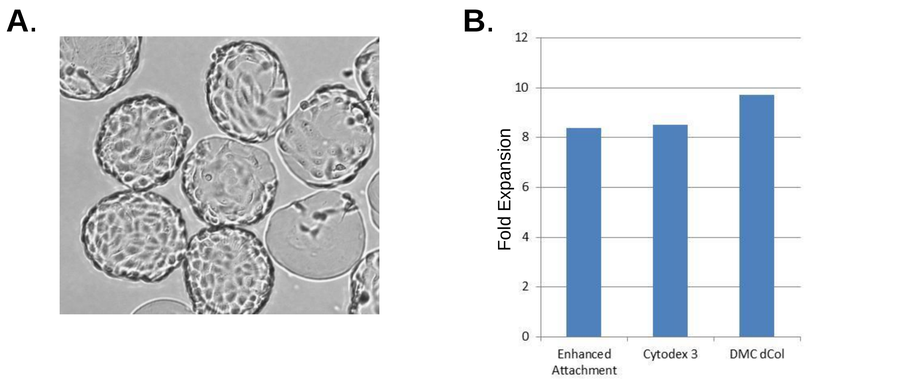


Figure 2. Vero cell growth on denatured Collagen dissolvable microcarriers was comparable to that observed for polystyrene and Cytodex control beads. (**A**) Phase contrast microscopy image of Vero cells after 4 days growth on denatured Collagen dissolvable microcarriers (DMC dCol). (**B**) Vero cells were grown on Corning enhanced attachment polystyrene, Cytodex 3, and DMC dCol microcarriers for 4 days with continuous agitation. Cells from enhanced attachment microcarriers and Cytodex 3 were harvested using trypsin. Cells were harvested from DMC dCol microcarriers using a digestion solution. Cell viability exceeded 90% for all microcarrier conditions (data not shown).

Figure 3.

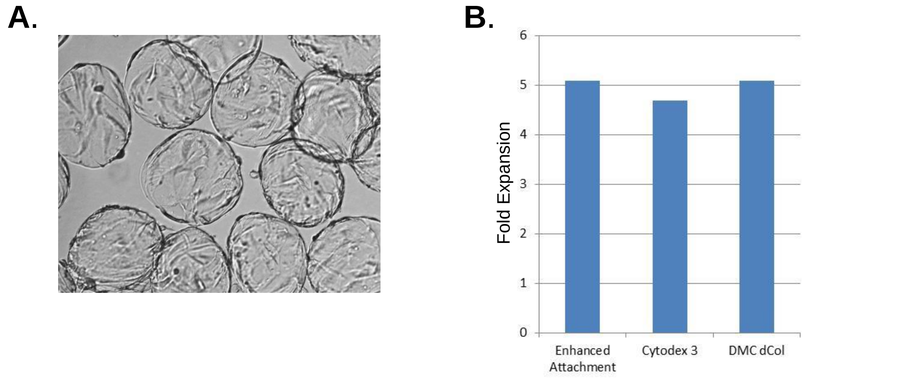


Figure 3. MRC5 cell growth on denatured Collagen dissolvable microcarriers was comparable to that observed for polystyrene and Cytodex control beads. (**A**) Phase contrast microscopy image of MRC5 cells after 6 days growth on denatured Collagen dissolvable microcarriers (DMC dCol). (**B**) MRC5 cells were grown on Corning enhanced attachment polystyrene, Cytodex 3, and DMC dCol microcarriers for 4 days with continuous agitation. Cells from enhanced attachment microcarriers and Cytodex 3 were harvested using trypsin. Cells were harvested from DMC dCol microcarriers using a digestion solution. Cell viability exceeded 90% for all microcarrier conditions (data not shown).

Figure 4.

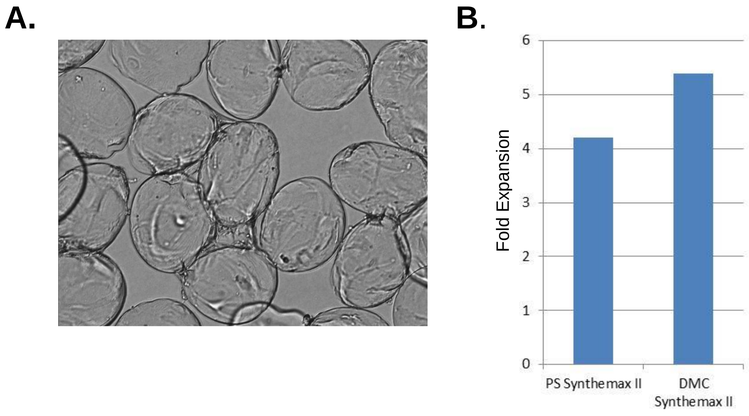
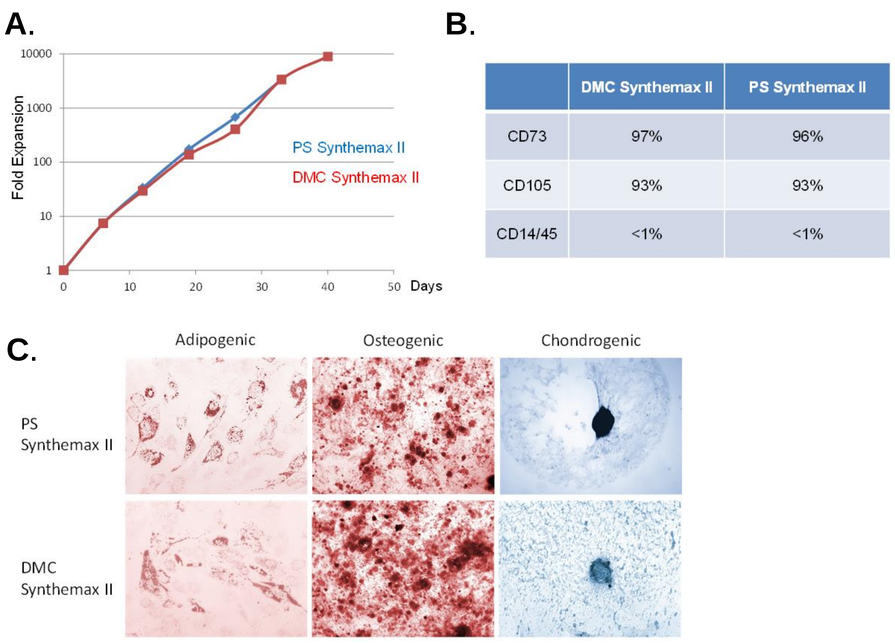


Figure 4. hMSC expansion on Corning Synthemax II dissolvable microcarriers was comparable to that observed for Synthemax II polystyrene control beads in serum-free medium. (**A**) Phase contrast microscopy image of hMSCs after 7 days growth on Synthemax II dissolvable microcarriers (DMC Synthemax II) in MesenCult-XF medium. (**B**) hMSCs were grown on Synthemax II polystyrene and DMC Synthemax II microcarriers for 7 days in MesenCult-XF medium. Cells from Synthemax II polystyrene microcarriers were harvested using TrypLE™. Cells were harvested from DMC Synthemax II beads using a digestion solution. For both microcarrier conditions, the cell viability exceeded 90% (data not shown).

Figure 5.



	DMC Synthemax II	PS Synthemax II
CD73	97%	96%
CD105	93%	93%
CD14/45	<1%	<1%

Figure 5. Comparable long-term expansion was observed for hMSCs on Corning Synthemax II dissolvable and polystyrene microcarriers in serum-free medium. (**A**) Cumulative hMSC expansion for 40 days on Synthemax II dissolvable (DMC Synthemax II) and polystyrene (PS Synthemax II) microcarriers in xeno-free Corning stemgro® hMSC medium is shown. At each passage, 80% of the culture was collected for cell enumeration and analysis. Fresh microcarriers were added to the remaining spinner flask cultures and cells colonized fresh beads via spontaneous bead to bead transfer. (**B**) Flow cytometry analysis of marker expression after 40 days growth in spinner flasks demonstrated a stable hMSC phenotype. **(C)** After long term culture on microcarriers, directed differentiation was induced using commercially available differentiation kits from Gibco. An efficient differentiation of the cells into adipogenic, osteogenic, and chondrogenic lineages was observed, indicating the maintenance of hMSC multipotency.

Figure 6.

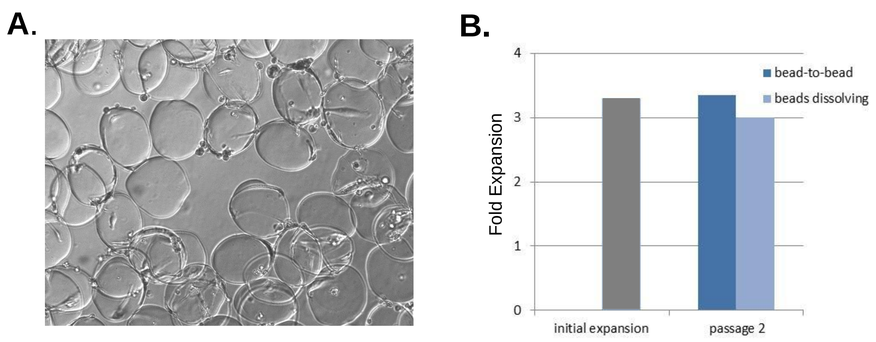


Figure 6. Comparable hMSC cell growth on Corning Synthemax II dissolvable microcarriers is observed when cells are passaged using either bead-to-bead transfer or bead digestion and re-seed. (**A**) Phase contrast microscopy image of hMSCs 18 hours post-harvest via beads dissolving and re-seeded onto fresh Synthemax II dissolvable microcarriers (DMC Synthemax II). (**B**) Expansion of hMSC grown for 7 days on DMC Synthemax II in serum-free medium is shown. Cells used for seeding were either: (1) attached to DMC Synthemax II microcarriers and new bead colonization was obtained by bead-to-bead transfer, or (2) cells were harvested from DMC Synthemax II by dissolving beads. For both microcarrier conditions the cell viability exceeded 90% (data not shown).

CLS-BPI-PST-016 10/15