Dissolvable Microcarriers for hMSC and hiPSC Production and Recovery

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Abstract

A new dissolvable microcarrier technology was developed that supports efficient cell production and recovery while eliminating the need for microcarrier cell separation. Dissolvable microcarriers are made from calcium cross-linked polygalacturonic acid polymers that are easily dissolved using a solution of EDTA and pectinase. To facilitate cell adhesion in serum-containing and serum-free applications, microcarriers are pre-coated with either porcine-derived denatured Collagen or Corning® Synthemax® II, a synthetic vitronectin peptide polymer. We demonstrate human mesenchymal stem cell (hMSC) and human induced pluripotent stem cell (hiPSC) growth on dissolvable microcarriers in spinner flasks and bioreactors. Upon microcarrier dissolution, nearly 100% of cells were recovered, and cells maintained their respective phenotype and differentiation capability.

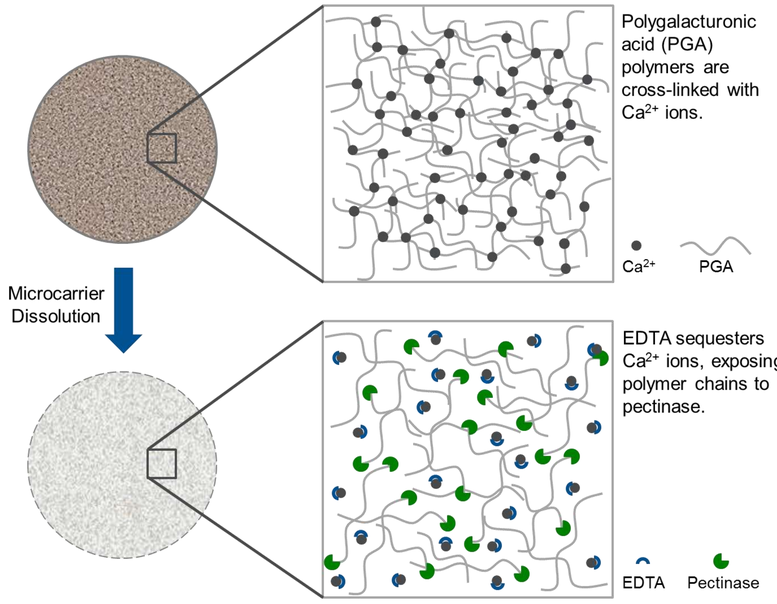
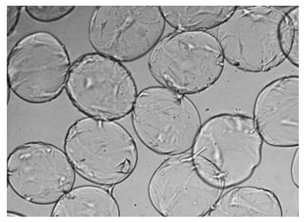


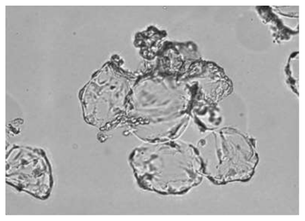
Figure 1: Corning Dissolvable Microcarriers.

Dissolvable microcarriers are made of polygalacturonic acid (PGA) polymer chains cross-linked via calcium ions. Microcarrier dissolution is achieved with the addition of EDTA (which chelates calcium ions and destabilizes the polymer crosslinking), pectinase (which targets degradation of the PGA polymer), and a standard cell culture protease (which breaks down cell and ECM networks). Microcarriers are completely dissolved within 5 to 10 minutes. Microscopy images show bead dissolution and hMSC release using a harvest solution of EDTA (Corning Cat. No. 46-034-CI), pectinase (Sigma Cat. No. P2611), and TrypLE™ (Thermo Fisher Cat. No. 12563011).

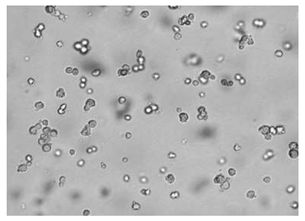
Surface area: 5,000 cm²/g **Density: 1.02-1.03 g/cm³** **Diameter: 240 ± 15 µm**



Pre-dissolution (t = 0 min.)



Dissolution (t = 1 min.)



Dissolution (t = 10 min.)

Cell Attachment | **Cell Expansion** | **Post-microcarrier Dissolution**

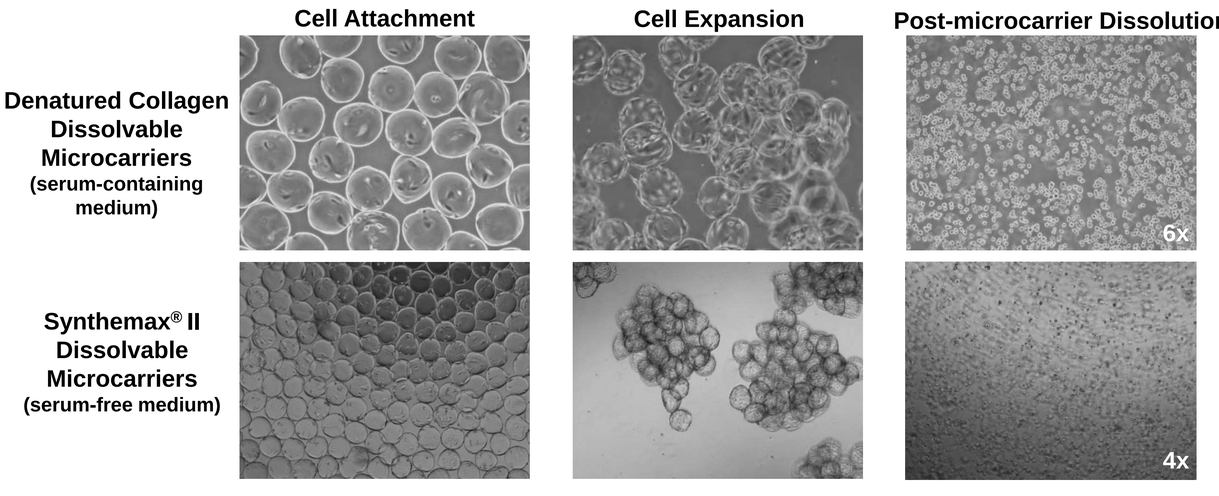
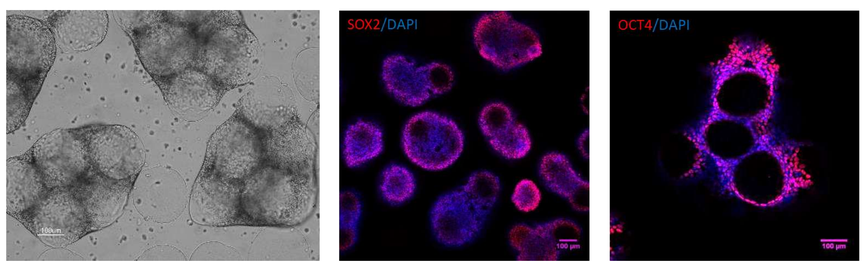


Figure 2: Human Mesenchymal Stem Cell (hMSC) Expansion on Dissolvable Microcarriers in Serum-containing and Serum-free Media in Larger Scale Cultures.

We achieved >25,000 cells/cm² (7-fold expansion) on 2g/L denatured collagen dissolvable microcarriers in serum-containing medium in 5L Sartorius Biostat® B Twin bioreactors. Continuous agitation was used during cell attachment (10 cycles of 5 minutes at 100 rpm, 20 minutes at 20 rpm) and expansion (120 rpm through day 4) phases. Microcarrier aggregate size was maintained through continuous gas sparging to maintain DO levels of 50%. Full-volume cell harvests were performed using a solution containing pectinase, EDTA, and TrypLE for 15 minutes with stirring at 100 rpm. The entire harvest process was completed by one operator in 1.5 hours and resulted in >90% cell recovery.

In addition, we achieved >40,000 cell/cm² (7-fold expansion) on 1g/L Synthemax II dissolvable microcarriers in serum-free medium in 1L glass spinner flasks. Intermittent mixing was used during cell attachment (24 cycles of 2 minutes at 22 rpm, 30 minutes at 0 rpm). Continuous mixing was used during the cell expansion phase (16, 22, 27, 32 rpm on days 1, 2, 3, and 4-7, respectively). Microcarrier aggregates were observed starting on day 3. Full-volume harvest resulted in complete microcarrier dissolution and a single-cell suspension.



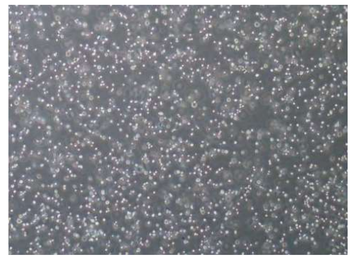
	Condition		
Parameters	hiPSC line 1 in mTesR1	hiPSC line 2 in mTeSR1	hiPSC line 1 in TeSR2
Number of Experiments	4	2	3
Exponential growth phase (days)	4	5	4
Specific Growth rate (day^{-1})	0.382 (±0.050)	0.270 (±0.002)	0.321 (±0.020)
Duplication time (days)	1.95 (±0.30)	2.60 (±0.02)	2.20 (±0.09)
Productivity (cells.ml^{-1}. day^{-1})	1.84E+05 (±25E+03)	1.08E+05 (±5.0E+03)	1.36E+05 (±7.5E+03)

Figure 3: Expansion of Human Induced Pluripotent Stem Cells (hiPSCs) on Dissolvable Microcarriers.

Two hiPSC lines were maintained on 1g/L Corning Synthemax II dissolvable microcarriers in either mTeSR™1 or TeSR™2 in replicate 30 mL spinner flask experiments. Parameters for the expansion of hiPSCs during 5 days of exponential growth phase are shown: the specific growth rate (day^{-1}), the duplication time (days), and the productivity rate (cells.ml^{-1}. day^{-1}). These parameters are the average of a set of independent experiments for each condition with the error representing the SEM.

Independent of culture condition, hiPSCs maintained high levels of pluripotency marker expression (OCT4, SSEA4, TRA1-60, SOX2) as detected by immunocytochemistry and FACS analysis. Further, cells recovered from dissolvable microcarriers retained their tri-lineage differentiation potential (data not shown). Dissolvable microcarriers supported high hiPSC productivities per day, and cell harvest via microcarrier dissolution with a solution of pectinase, EDTA, and Accutase® resulted in >95% recovery of viable cells within 5 minutes.

MSCs on Dissolvable Microcarriers Pre-dissolution



Recovered MSCs Post-microcarrier Dissolution

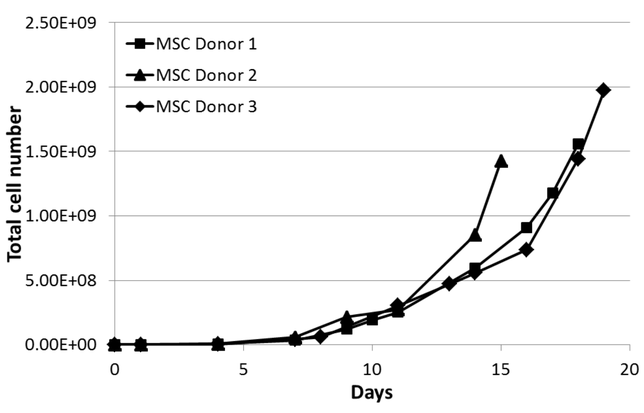


Figure 4: Mesenchymal Stem Cell (MSC) Expansion on Denatured Collagen Dissolvable Microcarriers.

MSCs were cultured using the 1L Scinus Cell Expansion system, to a total of 2 billion cells. Using Scinus's volume expansion capabilities and fresh microcarrier addition, cells were thawed and expanded >10 population doublings on dissolvable microcarriers in 15-19 days.

The total cell population was harvested by one operator in one hour, resulting in >85% recovery of high viability (>95%) cells. Dissolution of the microcarriers using a solution of pectinase, EDTA, and TrypLE resulted in a single cell suspension after harvest.

Using the Scinus closed bioreactor system and dissolvable microcarriers, we were able to culture and recover high cell yields that were not previously obtainable within one bioreactor system, greatly reducing the cost for cell therapy production by lowering the operator time, medium expenditure, and clean room requirements.

Conclusions

- The attachment and growth of hMSCs and hiPSCs on Corning dissolvable microcarriers was demonstrated in serum-containing and serum-free media in spinner flasks and bioreactors.
- In all applications, efficient recovery of high viability cells was achieved via microcarrier dissolution in a solution of pectinase, EDTA, and cell culture protease.