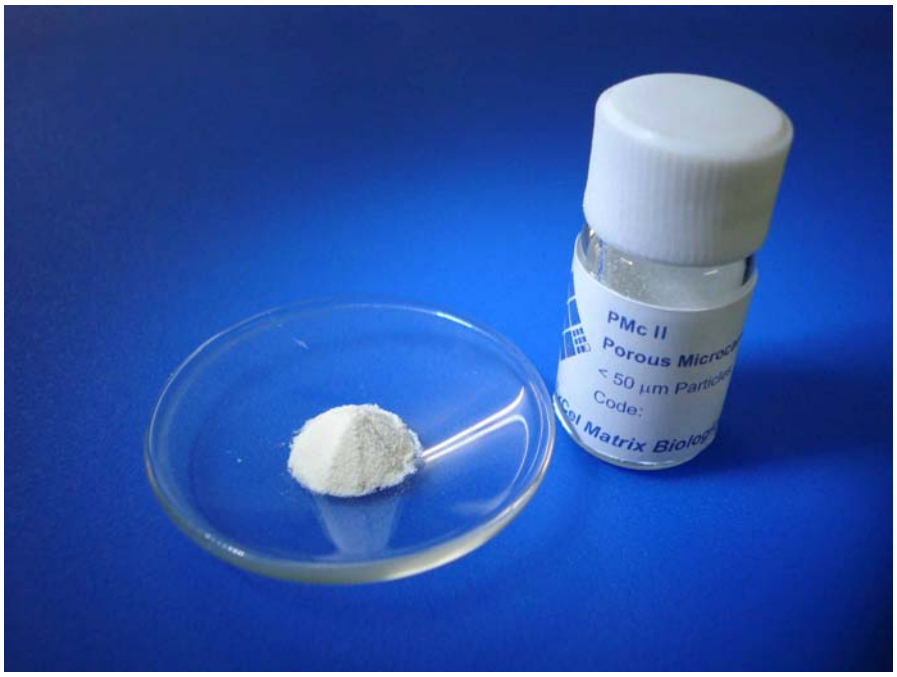
ExCel Matrix Biological Devices P. Ltd.
Hyderabad

3D Cell Culture
Microcarrier Culture
Cell Culture Bio-Assays
ExVivo Organ Model
Tissue Engineering

Porous Microcarrier PMc®

A Biodegradable Microcarrier



Microcarrier was developed in 1967 for viral vaccine manufacturing[1]. It remains as most reliable large scale manufacturing option till date. Microcarrier method can be scaled up easily, however cell yield (number of cells/mL) remains same as conventional methods like Roux bottle and roller bottle. Low product yield as consequence, very often do not justify large investment on bioreactors and utility needed to adopt microcarrier technology for viral vaccine manufacturing[2].

To improve cell yield by increasing the amount of microcarrier/mL is limited due to increase in collision among microcarrier, bioreactor impeller, baffles and walls leading to cell death.

Porous microcarrier, PMc® on the other hand protects cells from collisions as it is soft and allow cells to grow inside the cavities of large inter-connected pores. It is therefore possible to achieve more cells and hence product yield.

Cells can be harvested, as PMc® is exceptionally susceptible to trypsin and there is no carry over microcarrier particles during inoculum development.

Surface area of PMc® is also significantly higher than normal microcarriers to allow high density culture under perfusion mode.

Collagenous and ECM based porous scaffolds are quite suitable for tissue engineering[4]. PMc® is designed for regenerative medicine (tissue engineering and stem cell) research and application development. PMc® is made of extra cellular matrix like amniotic membrane, a well proven biomaterial from primary cell phenotype maintenance and clinical application point of views. PMc® has merged the advantages of microcarrier and bioreactors on single platform through ECM Analog® technology.

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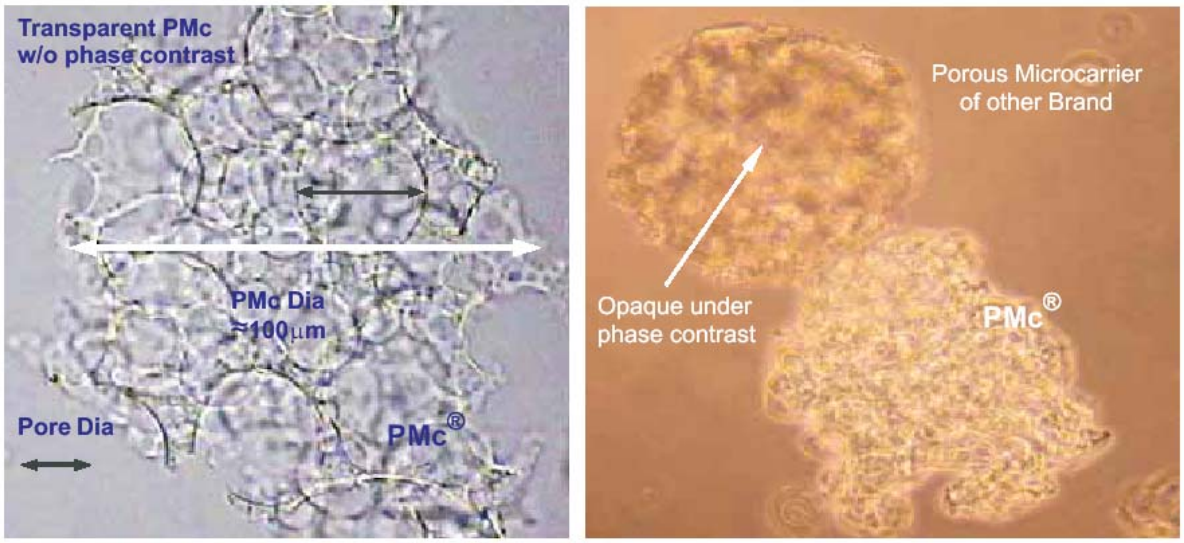
A Novel Porous Microcarrier PMc®

Based on Extra Cellular Matrix



PMc® as observed under normal microscope

Using Porous Microcarrier PMc®

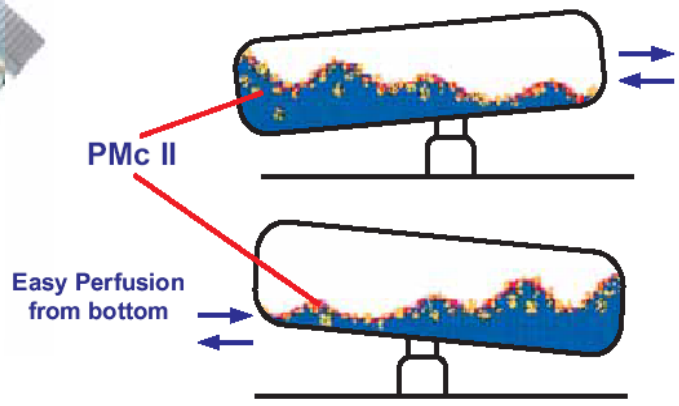
PMc® I

Density > Culture media
Sizes 50 to 100 µm

Sensitive to Microcarrier
Concentration, Agitation & O_2

PMc® II

Density ≤ Culture media
Sizes 50 to 100 µm



Little or no Limitations for
High Density Culture

Spinner Bottle and Shaking Bag Cultures

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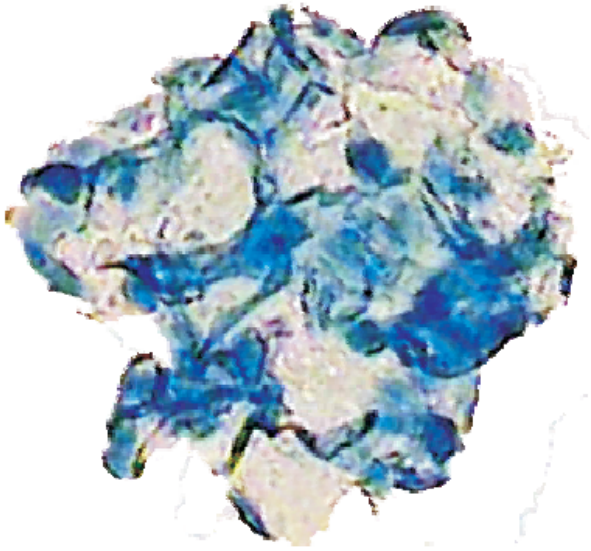
Porous Microcarrier PMc®



PMc® I with growing cells methylene blue stained

Applications and Highlights

- High Density cell culture for viral vaccine manufacturing.
- Recombinant protein manufacturing.
- Culture inoculum preparation at high density.
- Culture stock maintenance at lower passage numbers.
- *In situ* cryo preservation of 3D cultured cells.
- Regenerative medicine and Stem cell culture.
- Artificial tissue regeneration (Skin, Cartilage etc.).

General Features

- PMc® is made of extracellular matrix and hence suitable for primary cell cultures.
- High surface to volume ratio for maximum cell growth/ support.
- Broadly procedure of 3D cell culture on PMc® is similar to conventional microcarrier.
- High density culture with perfusion.
- Cell harvesting by trypsin in 3 minutes.
- PMc® is transparent for easy microscopic observation without phase contrast.
- Cells can be stained with methylene blue in Ca++, Mg++ free PBS to increase contrast against PMc® matrix with or without fixation.
- PMc® is suitable for culture in spinner bottles and Wave bioreactor.
- PMc® diameter is kept smaller than conventional microcarrier on purpose.
- PMc® II density is <1, hence,
  - PMc® II can float in medium without agitation.
  - Little or no O2 limitation with PMc® II floating at airliquid interface.
  - Easy perfusion from the bottom of culture vessel as PMc® II remains floating with cells on top at the air-liquid interface.

Precautions and Notes

1. Keep the total volume of medium as low as possible to increase cell attachment at the time of cell inoculation similar to conventional microcarrier.
2. Ensure that there is no residual protease enzyme in the cell suspension used for inoculation of PMc®. Protease enzyme will dissolve the ECM Analog®.
3. Maximum amount of PMc/mL of culture can be 10 mg/mL. Though much higher amounts are feasible to achieve higher cell yields but such cultures will essentially require perfusion to support cells and remove toxic metabolites from cultures[5].
4. Cell concentration for inoculation need to be adjusted depending upon duration of experiment, cell multiplication etc. For example, if cell have low multiplication potential or not required to multiply, cells may be inoculated in high concentration (< 0.1 million to a few million cells/ mL culture volume), i.e. typical saturation density of monolayer cultures.

References

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2. Levine DW, et al. Biotechnol. Bioeng. 21, 1979, pp 821-845.
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5. Nahapetian AT, et al. J. Ce ll Sci. 8, 1986, pp 65-103.